Medical Lib

BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

Vol. XXXII—No. 362] BALTIMORE, APRIL, 1921 [Price, 50 Cents

CONTENTS

	PAGE
The Dangers to Life of Severe Involvement of the Thorax in Rickets. (Illustrated.) By Edwards A. Park and John Howland	101
Diphtheria Bacillus Carriers. A Report of Conditions Found in an Orphan Asylum. By W. L. Moss, C. G. Guthrie and J. Gelien	109
Studies with Lecithin and Cholesterin in Relation to the Antihemolytic Property of Human Serum. By Hallie M. Clark and Frank A. Evans	113
Variations in the Bacterial Flora of the Upper Air Passages During the Course of Common Colds. By Arthur Bloomfield	121
Hemolytic Influenza Bacilli. By T. M. Rivers and Erida L. Leuschner	130
Experimental Observations on Bone Marrow. (Illustrated.) By George B. Wislocki	132
The Resignation of Dr. William Sydney Thayer	135
Minute on the Resignation of Dr. Thayer	135
Books Received	136

Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

THE DANGERS TO LIFE OF SEVERE INVOLVEMENT OF THE THORAX IN RICKETS

By Edwards A. Park and John Howland

The thorax is affected like all other parts of the skeleton in the course of severe and prolonged rickets, and frequently becomes the seat of most extensive deformities. A mild degree of involvement of the thorax in the rachitic process is itself of little or no importance. More severe involvements, however, constitute a veritable menace to life itself. The object of this paper is to call attention to a little recognized but very definite symptom-complex which is accompanied by characteristic autopsy findings. The symptoms and pathological changes in question are the direct result of the diseased condition of the thorax. We also wish to discuss the mechanism by which these symptoms and pathological changes are produced and life is threatened.

The material, which forms the basis for our study, consists of 32 children between the ages of 8 months and 4½ years. All the children exhibited well-marked rachitic deformities of the thorax. In the case of six of them, however, the rachitis had not progressed far enough to produce great weakening of the thoracic wall and in four there had occurred healing or at least thickening of the ribs to an extent sufficient to make the deformed thorax rigid. Excluding these ten cases, which served only for the study of the deformities of the thorax, there remain 22 cases. These 22 cases form the actual basis for this report. In all of them there were present not only the characteristic deformities of the thorax but also the essential element, great weakening of the thoracic wall, and in all there were the characteristic train of symptoms and X-ray changes in the lungs, to be enumerated later. The youngest of the 22 children was but 8 months old. Only one child (aged 3¼ years) was more than 2½ years of age. The average age was 16 months. Thirteen were boys, nine girls. It so happened that all were colored. Although rachitis reaches grades of development in the black race in America rarely encountered in the white race, nevertheless, the condition described occurs as well in white as in black children. We have seen well-marked examples in white children in both this country and France. It is important to note that all 22 children were greatly under weight. One weighed 64 per cent under the normal standard for the age. Their average weight was 46 per cent under the average weights for healthy children of corresponding ages. All 22 children also were undersized. The body length averaged 88 per cent of the average for the corresponding ages. It is interesting to observe that the circumference of the chest was proportionately less than the length of the body. It was 83 per cent of the average for healthy children of corresponding ages. The circumference of the head averaged 96 per cent of the average of the standard measurements for the corresponding ages.

All 22 children also showed clinical evidence of rickets in parts of the skeleton other than the thorax. The deformities of the head and extremities were not particularly striking in all instances, however, and not in proportion to the deformities of the thorax. The majority had multiple fractures of the extremities, in a number of them not recognized until examined with the X-ray. A rhinitis with mucous discharge and resulting stenosis of the nasal passages was present in the majority of the children. In some, undoubtedly, it played a part in the causation of the inspiratory collapse of the thorax and hence in the development of the respiratory condition.

When viewed from in front, the deformed thorax appears smaller than it actually is, in part because of its peculiar shape, presently to be described, in part because of its situation between the disproportionately large rachitic head and distended abdomen. Roughly the deformed thorax may be likened to a blunt wedge, the sternum and adjacent portions of the costal cartilages corresponding to the narrow end of the wedge. The peculiarity in its shape is due to the fact that the antero-lateral portions have sunk inwards with the production of broad hollows, the deepest parts of which correspond to the sites of the costochondral junctions (Figs. 1 and 2). At the end of expiration, these depressions extend in a general way from the midaxillary line behind to within 2–3 cm. of the sternum, sometimes including, sometimes failing to include the nipple, and from the third or fourth rib above to the ninth below, where the thorax usually begins to flare outward over the abdomen. The upper part of the depression, which is narrow, is usually covered by the pectoralis major muscle. The deepest part lies at the sixth or seventh costochondral junction. The depression on the right side is in most cases larger and deeper than the one on the left, probably because the heart furnishes support to the thoracic wall on the left side. In the less severe forms there may be no depression on the left side but only flattening of the normal arch. The posterior angles of those ribs entering into the formation of the depression are much more bent than normal. Together they form a rounded ridge-like elevation 3–4 cm. broad, which projects laterally and sharply separates the back from the antero-lateral surface of the chest. Anteriorly this ridge slopes downward into the floor of the depression and forms its posterior boundary.

If the child can be made to sit up, it is at once apparent that the clavicles are exceedingly prominent. They are much bowed and stand out unusually far from the base of the neck. In a considerable number of cases they are the seat of fracture. The manubrium is prominent and appears to be elevated, as indeed does the whole thorax. In consequence of the high position of the thorax, the neck in front seems unusually short (Fig. 3). The sternum is thrust forward and is not infrequently curved or bent. Its inclination downwards and forwards is often much exaggerated. The prominence of the sternum, which might be compared to the inverted hull of a boat, is due to the fact that the ends of the ribs on the two sides of the chest are closer together than normal as the result of the collapse of the thoracic wall at the sides. A great increase in the arch of the costal cartilages is thereby produced. Occasionally on one or on both sides the costal cartilages are so greatly arched that those parts of them immediately adjacent to the sternum bow forward in front of the sternum and convert the latter into the floor of a groove. The manubrio-gladiolar junction often forms an obtuse angle pointing downward. In several of our cases the gladiolus was so inclined forward that it formed an angle of about 10° with the vertical, and in one instance an angle estimated at 25°. The xiphoid often points slightly backwards. The costal margins composed of the conjoined cartilages of the lower ribs and the ribs themselves usually flare outwards. On account of the hollows at the sides and the flaring of the lower margins the thorax when removed from the body bears a resemblance to a corset.

As already indicated, the thorax is not symmetrically deformed; indeed it is often markedly asymmetrical. The lateral depressions are never the same on the two sides. The broader the depression, the narrower the anterior surface of the thorax on that side and the sharper and more prominent the ridge made by the posterior arches of the ribs behind; the deeper the depression, the greater the flare of the costal arch below. The sternum itself often deviates from the median line and slopes toward one side or the other. The costal arch is always higher on one side than on the other and the flaring on the two sides unequal. If the child has sat, the vertebral column always exhibits lordosis in the interscapular region with kyphosis below and often well marked scoliosis. The back is rarely flat. If one of the lateral depressions is particularly wide and deep, the ribs involved pass backward and outward from the vertebral column instead of outward, causing the back on that side to project posteriorly. Moreover, the deeper the lateral depression in front, the narrower the back on that side behind (Fig. 4).

When fractures of the ribs are present, the distortion of the thorax may be greatly increased and the resulting deformities much more difficult to analyze. When the fractures are situated a short distance behind the costochondral junctions, small pits 1–1.5 cm. in depth are often formed. When the fractures are near the posterior angles of the ribs, these angles lose their rounded character. Since one rib derives support from another, often several ribs become fractured in series and, inasmuch as the points of fracture usually occur in line with each other, the resulting deformities appear as sharp ridges running obliquely down the side of the chest with a depression in front of them. It is of course impossible to do more than indicate the bizzare forms which the thorax may assume under the influence of the rachitic process with its resultant fractures.

When inspiration takes place in the deformed thorax just described, almost every deformity which has been mentioned becomes intensified. The depressions at the sides lengthen, broaden and deepen. The floor is carried backward and inward toward the vertebral column, in severe cases from 1–1.5 cm. and even 2 cm. As the lateral depression en-

larges, the posterior angles of the ribs involved narrow, and the whole posterior costal arch undergoes a displacement backwards. The prominence formed by the sternum and costal cartilages narrows as if squeezed together at its base. The manubrium moves slightly forwards and upwards. The lower end of the gladiolus remains stationary or is drawn backward; in some cases it moves slightly forwards as in older children with chicken-breast. Often the sternum describes see-saw movements with respiration, or with each inspiration the middle part of the sternum bows forward beyond its upper and lower ends. In general, it may be said that every hollow, every eminence, every peculiar curvature or inequality present during expiration becomes increased during inspiration.

The moment that the inspiratory effort is complete, the whole thorax snaps back into the expiratory position as if released from a spring.

If measurements of the circumference of the thorax are taken, it will be found that the circumference at the level of the nipple either increases to the extent of only 1 or 2 mm. during inspiration or is not increased at all or undergoes an actual loss (Fig. 5). In reality, however, it is impossible to obtain correct measurements of the variations in the circumference of the thorax during inspiration, because the tape bridges over the hollows of the sides. It is difficult to detect, anywhere in the diseased thorax, movements of the ribs occurring during inspiration which would bring about an actual increase in intrathoracic volume save the feeble upward and forward movements of the upper part of the thorax indicated by the slight movements in those directions of the manubrium. The evidence of the loss of intrathoracic space during inspiration in the collapse of the thoracic frame at the sides is so apparent as to leave little doubt that, the movement of the ribs alone considered, the net result of any given inspiration must be actual loss. Since the child remains alive, however, there must be some mechanism available through which the capacity of the thorax is increased with each inspiration. That mechanism must lie in the diaphragm. We shall return presently to the discussion of the diaphragmatic function.

The deformities and unusual movements of the thorax just described are brought about because the bony thorax has lost its rigidity. This loss of rigidity is the direct result of the failure of calcium deposition and, very probably, also, of resorptive processes in the bones themselves set in operation by the disease. The ribs, normally composed largely of inorganic material, firm and unyielding, become composed chiefly of organic material and are soft and yielding. The shafts of the ribs of some of the children, who have died, offered about the same resistance to bending as if they had been composed of pasteboard. In these severe cases fractures are almost regularly present. In one case we counted twenty. These further greatly impair the rigidity. The most extreme weakening at any portion of the costochondral arch develops at the costochondral junctions.

Under normal conditions the cartilage and shaft of the rib meet as do two accurately matched beams. The opposed ends of cartilages and ribs are slightly uneven and are to a certain extent mortised into each other. The union between cartilage and shaft is made firmer by the columns of calcified intercellular substance which, forming in the terminal zone of cartilage, extend like so many nails into the substance of the shaft. The normal costochondral junction is so strong that, when an attempt is made to break it, it is the shaft of the rib which buckles under the strain. In rickets a zone composed of osteoid tissue (bone tissue devoid of its normal content of calcium), connective tissue, blood vessels and degenerated and undegenerated cartilage in the form of bands and islands, gradually forms between the cartilage and shaft. This heterogeneous mass of tissue, known as the transitional zone or metaphysis, has no rigidity and is capable of offering about the same degree of resistance to any deforming force as connective tissue. As a result of forces acting upon the thorax, the weak metaphysis begins to yield almost as soon as it begins to form. The costal cartilages, wholly unaffected by the disease, retain their normal elasticity and, when forced inward during inspiration, tend always to spring back into their normal positions. The diseased ribs, however, never having possessed an elasticity at all comparable to that of the cartilage, lose the greater part of what was formerly theirs. As the result of the continuously repeated strain, to which they are subjected by inspiration, they become bent further and further inwards until finally their extremities lie internal to the cartilage. In the most severe forms of rachitic involvement of the thorax the costochondral junctions become so deformed that the end of the cartilage is no longer joined to the end of the shaft but to the outer side of the end of the shaft (Fig. 6). When the costochondral junctions have become affected to this extent, all rigidity has long since disappeared; indeed the shaft and cartilage have become so loosely attached to each other that a considerable degree of motion can readily take place between them. In this state the costochondral junction really constitutes a false joint.

The forces that operate to produce deformity in the thorax are the negative pressure in the thoracic cavity existing during inspiration, the elasticity of the lungs, the pull of the diaphragm and of the various voluntary muscles passing to the thorax from the abdomen and neck. To this list of forces might be added two of less importance, gravity and intra-abdominal pressure. With the exception of gravity, the forces mentioned are applied either solely or in by far their greater part during inspiration. The normally curved, strong, resilient thorax of the healthy child withstands them completely. When the negative pressure within the normal thorax reaches extreme heights during inspiration as in laryngeal stenosis, it is always the costal cartilages of the lower ribs and the lower part of the sternum which yield and are sucked backwards. The ribs and costochondral junctions unaffected by disease are capable of withstanding great strain. The diseased ribs and costochondral junctions which form the wall of the rachitic thorax cannot do so. The forces brought to bear upon them gradually cause the shafts of the ribs to sink inwards anteriorly, carrying the cartilages with them. The

muscles of the neck attached to the first and second ribs and clavicles pull these bones upwards. The abdominal muscles draw the lowermost ribs downwards. Intra-abdominal pressure, if in excess of the normal, forces the lower aperture of the thorax outward. The weakened thorax has become the prey of every force exerted upon it.

Reference has already been made to the fact that the circumference of the thorax is diminished, remains stationary, or is increased very slightly during inspiration. Although it is impossible to determine the point, it is highly probable that as the result of thoracic respiration alone an actual loss of intrathoracic space would occur during inspiration. It is the action of the diaphragm that makes life possible. But the diaphragm can act to advantage only when its points of attachment remain firm. In the weakened thorax of rickets the diaphragm expends part of its energy in drawing its own points of attachment inward. In so doing it diminishes the extent of its descent and impairs by just so much its power to increase intrathoracic space. This is not all. Owing to the deformity of the thorax, the diaphragm must tend to remain even in expiration in a state of partial inspiratory contraction. When inspiration takes place, it must commence its contraction from a position of partial contraction and is able, therefore, to accomplish only the last part of the work which, as a muscle in a state of normal relaxation at the outset, it would be capable of accomplishing. It is quite possible also that the contractile power of the diaphragm is impaired as a result of the rickets itself.

The changes just described in the thoracic wall must inevitably lead to changes in the thoracic contents. When the thorax is opened, attention is immediately attracted to two striking conditions (Fig. 7), the knuckle-like enlargements at the costochondral junctions which project a considerable distance into the thoracic cavity, and the grooves, one on the external surface of each lung not far from the anterior border, running in a direction from above downward, outwards and backwards. If the lungs are pulled forward into their anatomical positions, the grooves on the surface of the lungs come to lie directly opposite the lines of the deformed costochondral junctions, and the relationship between the two is clearly shown by the presence of depressions in the grooves exactly corresponding to the costochondral prominences themselves. In reality the depressions are moulds of the prominences. When the lungs are removed from the thorax, it is seen that they are far too small for the size of the child. The right lung is usually proportionately smaller than the left. It can then be seen that the grooves divide the external surface of each lung in such a way that roughly one quarter lies in front and three-quarters behind. That part of the lung in front of the groove is for the most part emphysematous, while that part lying behind it is generally dark bluish in color and of a firm consistency. Obviously it is atelectatic. It is not entirely atelectatic, however, for interspersed with the collapsed pulmonary tissue is tissue in a state of emphysema. In some cases the greater part of one lobe is atelectatic. On section through the lung, it can be seen that the floor of the groove is composed of atelectatic tissue and that the thickness of the lung at the groove has been reduced to 2–3 mm. Further, the part lying in front of the groove, though chiefly of emphysematous tissue, contains scattered areas of atelectasis, while behind the grooves atelectasis so predominates over emphysema that the greater part of the lower lobe appears to be collapsed (Fig. 8). Areas of lobular pneumonia are almost always present. They are not large and frequently cannot be recognized on gross examination. The cut surface almost invariably exudes serum, showing the presence of edema. No pleurisy is present. In some cases, however, adhesions are found between the visceral pleura and the sharp points at the summits of the knuckle-like enlargements of the costochondral junctions (Fig. 6). These points are formed by the projection of the pointed shafts of the ribs through the rachitic metaphyses. The adhesions are produced by the irritation of the sharp bony points against the pleural surface. On microscopic examination of the lungs atelectasis is the predominating and essential condition in the more solid parts. Evidences of inflammation can usually be found, but large areas of the lungs are not the seat of pneumonic consolidation.

As a result of the deformity, the thorax becomes divided into two communicating chambers (Figs. 9 and 10), an anterior one bounded in front by the sternum and abnormally arched costal cartilages and at the sides by the projecting walls of deformed costochondral junctions, and a posterior one bounded by the rest of the thorax. In the anterior chamber the heart is imprisoned. The apex of the heart always lies against the barrier of the costochondral junctions on the left, usually at the level of the fourth or fifth rib. The distended right auricle, forming the right border of the heart, reaches to within a short distance of the costochondral junctions on the right side.

On removal of the heart and exposure of its cavities, that of the left ventricle appears to be normal in size and to have normally developed internal musculature, but that of the right ventricle often appears dilated and hypertrophied (Fig. 11). The latter stands open and is half again as large as the cavity of the left ventricle. The columnæ carneæ are thick and flattened out against the ventricular well. Though the weight of the body is considerably less than the average for the age, the weight of the heart may equal or even exceed the average for the age. The wall of the left ventricle is of normal thickness; the wall of the right ventricle is frequently much thickened. Hypertrophy of the heart is, therefore, present, and the part giving evidence of it is the right ventricle. In one of our cases there was hypertrophy of the left ventricle as well. Upon the surface of the liver can invariably be seen the imprints of the 7th, 8th and 9th costal cartilages impressed through the diaphragm. The intestines are usually distended.

A number of months of progressive rickets must elapse for the costochondral junctions and shafts to become so weakened that the characteristic clinical condition develops. The clinical picture in well marked cases is as follows: The child lies on the back with arms at the sides and legs semiflexed and rotated outward (Fig. 1). The external rotation of the legs,

due to the laxity of the muscles and ligaments and probably also to curvatures in the bones themselves, is so extreme that, though flexed, the knees lie in contact with the bed. The child lies remarkably still, never attempting to change the position of the body and only occasionally turning the head or shifting slightly the extremities. The head shows the marks of rickets, as do the extremities, and the forehead is frequently covered with sweat. The large head appears as if placed directly on the thorax. The chin rests against the upper part of the chest and a deep fold in the soft parts alone indicates the neck. The expression is anxious. The mucous membranes may be slightly cyanotic. The dyspnea is extreme; the respirations are from 60 to 100 a minute—and in one case 150. They are accompanied by dilatation of the nostrils and an expiratory grunt. A cough occurs from time to time, usually singly, separated from succeeding coughs by one or more respirations. Sometimes, however, the cough comes in short paroxysms which seem to distress and terrify. The respirations are shallow. With each inspiration the thorax contracts. When the child coughs its collapse is doubled. As the thorax sinks in with each inspiration, the abdomen expands. Indeed the appearance suggests that the contents of the thorax are being rhythmically squeezed into the abdomen rather than that the lungs are being inflated and the normal process of respiration is taking place.

On account of the almost continuous movement of the thorax, especially marked and jerky in the region of the costochondral junctions, the determination of the location of the apical impulse of the heart is exceedingly difficult. It may be impossible to locate it. Usually it can be seen in the fourth or fifth spaces or in both about 1 cm. beyond the nipple line, often in or just beyond one of the depressions at the costochondral junctions. In some cases the apex impulse is strong and visible over rather a wide area and can be felt[1] as well as seen. There may be marked epigastric pulsation, or pulsation over the entire left side of the precordium. It is impossible to determine the location of the left border of the heart by means of percussion on account of the proximity of the enlarged costochondral junctions and impossible to determine the right border by that means with any degree of accuracy, not only on account of the changes in curvature and the abnormal character and asymmetry of the thoracic wall already described, but on account of the abnormal conditions in the lungs themselves. If, however, the costochondral junctions on the two sides lie close together, it may be assumed that the right border of the heart is close to the wall formed by the costochondral junctions of the right side, that is, the heart occupies the entire space between them. The action of the heart is very rapid. Unless cardiac disease unrelated to the rickets is present, the sounds are clear. We have never observed an accentuation of the pulmonic second sound which could be regarded as pathological.

In the examination of the lungs it is difficult to obtain accurate information concerning pathological conditions by means of percussion on account of the abnormal physical conditions in the thorax itself. Changes in percussion resonance are always present and easily demonstrated, but are not necessarily produced by changes in the lungs. Usually an impairment of the percussion note can be found in some portion of the chest, but in our experience it is never great. In not one of our cases was there well marked dulness. The breath sounds also are subject to great variation in different parts of the chest. In the lateral depressions they are often of an exaggerated vesicular character. Invariably in some parts of the thorax the breath sounds are greatly diminished and may be scarcely audible. The respiratory murmur may be higher pitched than normal, expiration may seem prolonged but loud bronchial breathing is never present. Râles can always be heard. They may be abundant or few, fine or moist, but are never evenly distributed throughout the lungs. They are most apt to be numerous over the bases of the lungs. The liver edge can be felt, usually 4 cm. or more below the costal margin. The spleen is often palpable.

The X-ray findings in the chest are characteristic (Figs. 12 and 13). The ribs look extraordinarily slender and have a porous appearance. They are bent and have lost their normal inclination and parallel arrangement. Fractures, even though present, are not evident. In cases of long standing rickets, in which the thorax has finally acquired rigidity, the ribs may appear greatly thickened. If scoliosis of the vertebral column is present, it is of course visible. The heart seems disproportionately large for the size of the thorax, often appearing enormous in comparison with the latter. On both sides of the heart are longitudinal shadows with indefinite margins 1–1.5 cm. wide (in X-ray plates of ordinary magnification). They extend downwards, at the same time curving slightly outwards, from a point at about the level of the second or third thoracic vertebra to the shadow of the liver and diaphragm below. The one on the right side begins near the shadow of the vertebral column above and is separated by a slight interval from the right border of the heart below. The one on the left side merges as it descends with the shadow of the heart or skirts the left border of the latter. The two longitudinal shadows are produced by the atelectatic lung beneath the costochondral junctions. If the costochondral junctions themselves cast shadows, as they do when healing has commenced, these also share in the formation of the longitudinal shadows. Elsewhere the lungs have an irregularly hazy appearance, due to the existence of scattered areas of atelectasis and also in some cases to scattered areas of an inflammatory nature. The lungs external to the longitudinal shadows never appear clear and never show the large dense clearly outlined shadows of complete consolidation. The

[1] Wherever on the chest wall the apex impulse of the heart may appear to be, however, there can never be any doubt in regard to the situation of the apex of the heart itself. The heart is always imprisoned in the space bounded in front by the sternum and cartilages and at the sides by the wall of costochondral enlargements which project into the cavity of the thorax. Beyond the barrier of the costochondral junctions the apex of the heart never passes. Any impulse which the heart can communicate to the chest wall by means of its apex is caused by the impingement of the apex against one of the costochondral junctions in the barrier.

X-ray findings in the thorax in these cases, though quite typical, are often erroneously considered to indicate tuberculosis.

The clinical picture just described greatly resembles that of pneumonia and is usually considered such. Yet the temperature is rarely greatly elevated, and is not infrequently normal (Fig. 14). In one of our cases the temperature was normal for the first seven days in the hospital. It began to rise only two days before death, reaching at that time 101° F. During the nine days of observation the respirations were never less than 60 and averaged between 80 and 100 to the minute. The leucocyte count also is low. In the 22 cases the leucocyte count varied between 4000 and 18,000 and averaged 11,000. In some of the severest cases the leucocyte count did not exceed 8000. Another fact of importance is that the symptoms remain but slightly modified after the evidences of infection have disappeared. The clinical picture just presented is caused by the loss of the rigidity of the respiratory frame, the result of the rickets. The infection is subsidiary; it furnishes the extra burden which the enfeebled thorax cannot bear.[2]

The sequence in the development of the condition seems to be as follows: The rachitic thorax loses its rigidity, yielding during inspiration simultaneously at costochondral junctions and shafts. As the rigidity of the thorax becomes diminished, the depressions at the sides, which at first were present only during inspiration, persist during expiration. As the bones progressively lose their elasticity, the dynamic deformity gradually becomes static as well. Thoracic respiration is at first slightly, later profoundly, affected. Coincident with the impairment of thoracic respiration diaphragmatic respiration is enfeebled. The efficiency of each inspiration, therefore, is diminished. A compensation is attempted in an increased frequency of respiration and in an increased force. But the greater the force applied, the greater the collapse produced. Nature's efforts in large part defeat themselves. The diaphragm pulls its attachments yet further inward, and the accessory muscles draw the bones to which they are fastened out of position without moving or giving stability to the thorax as a whole. Owing to the progressive reduction in the size of the thorax and its loss of power to expand, the lung begins to become atelectatic. One of the parts early affected is that pressed upon by the costochondral junctions. As the pathological process progresses, the atelectasis increases; the pressure in the pulmonary circulation rises; and hypertrophy of the right heart follows. If the rigidity of the ribs becomes still further impaired, a point is reached at which the disabled respiratory machine is just sufficient to perform its work. If now a slight additional burden is put upon it in the form of an infection, it becomes insufficient. Nature resorts to the only expedient possible; the frequency of respiration becomes enormously increased; the clinical condition above described ensues.

The course in such cases as we have been discussing is subject to great variation. In one group of children the respiratory symptoms and distress became progressively worse until death intervened. In another group the condition remained essentially unchanged over a long period. From time to time the temperature would rise to 102° or 103° F. without apparent cause or any increase in the number of white blood cells. It would remain elevated for a day or two, and then fall to normal again. With the rise in temperature, the respiration would become more rapid and the respiratory distress more acute. Death often seemed to impend, but with the fall in temperature the condition of the child would again ameliorate. This state of affairs, characterized by alternating exacerbation and decline of symptoms, has continued for weeks. Such children after spending several weeks in the hospital improve sufficiently to leave, but in a short time are readmitted in the same dangerous condition as before.

If a child does not die, he may gradually improve, even though the rickets continues and lime salts are not deposited in the bones. The shafts of the ribs and the costochondral junctions ultimately may acquire sufficient strength to withstand the forces exerted upon them. Given time, osteoid tissue is produced in such quantity both in the cancellous tissue, under the periosteum and in the metaphysis itself as to supply the requisite rigidity. Even though the costochondral junctions remain weak, respiration will take place in a normal or approximately normal manner provided the shafts of the ribs acquire sufficient strength. Ultimately a rigidity may be furnished the ribs through an overproduction of inferior material. It is due to this fact that we do not often see cases, such as have been described, in children older than 2½ years. Extreme deformities of the thorax are common enough in children 3 and 4 years of age, but the deformed thoraces will almost always be found to be rigid, and the bones of such children, not only the ribs but also the bones of the extremities, will be seen to be enormously thickened when studied by means of the X-ray. The children of our series whose thoraces were the weakest were children from 8 months to 1½ years old. *For the comprehension of the condition it is necessary to realize that deformity and normal thoracic function are compatible, in other words, that the element of danger is not the deformity but the loss of rigidity of the thoracic wall.*

With the aid of cod-liver oil calcium salts can be deposited in the rachitic metaphyses and shafts and a true healing of the disease accomplished. In spite of this, however, the deformities will persist for months or years. But gradually, we cannot say how rapidly, the deformities of the thorax are outgrown. In adults and in older children malformations of the thorax, such as we have described, are never seen. Changes in the extremities such as bow-legs and knock-knees are common enough in adult life. It is rare, however, to find a rachitic adult showing more than slight evidence of previous thoracic deformity. The pigeon-breast of older children and adults is usually a late development for which continued obstruction in the upper respiratory tract rather than rickets is responsible.

In regard to the mortality we cannot say a great deal. Out of a series of 22 well marked cases studied in the hospital, we

[2] See legend explaining Fig. 5.

know that 11 have died.[3] It is probable that others of the 22 children discharged have died outside the institution as the result of the rachitic involvement of the thorax. The mortality in the severely affected cases is undoubtedly exceedingly high.

Rickets is often looked upon as a disease that in itself does not threaten life. It does threaten life, however, and may be the direct cause of death when it deprives the thorax of its rigidity. It increases the danger to life from all respiratory infections, *e. g.*, tuberculosis, pneumonia, at that moment when it causes the thorax to yield during inspiration.

Treatment must be prophylactic. Such extreme rachitic changes as we have described should never develop. To bring rickets to a sudden termination is in the present state of our knowledge out of the question. To offer mechanical support to the yielding ribs through orthopedic devices seems impracticable. At present the only course is to administer cod-liver oil in moderate doses, to avoid infection and those things which give rise to abdominal distention.

Before concluding this paper, we wish to point out that we have found in the rats rendered rachitic by means of faulty diets[4] a condition identical with that just described in children, the subjects of the most severe rachitis. In the course of severe rickets in the rat, growth becomes greatly inhibited. The weight falls far behind the weight of the healthy animal and finally remains stationary. Growth in length is also much retarded. Multiple fractures of the ribs develop. As a result of the weakening effects of the disease upon the bones, the shafts of the ribs and the costochondral junctions lose their rigidity. The thorax collapses at the sides and the costochondral junctions become deformed and displaced well into the interior of the thorax. The costal cartilages join the shaft often at right angles and are attached not to the ends but to the outer sides of the shafts near the end. The deformed costochondral junctions of the rachitic rat are the deformed costochondral junctions of the rachitic child in miniature. The sternum and adjacent portions of the cartilage are thrust forward and the sternum acquires an abnormal forward inclination as it descends, exactly as in the human being. As the result of the displacement of the costochondral junctions inward, the thoracic cavity becomes divided into the two chambers already described (Fig. 15). The heart is imprisoned in the anterior chamber, which it completely fills. The lungs are partially collapsed and show bands of atelectasis in those parts subjected to the pressure of the costochondral junctions, and scattered areas of atelectasis elsewhere.

With each inspiration the whole lateral wall of the thorax sinks forward. Respirations are enormously increased. In one rat, the subject of advanced rachitis of the thorax, the respirations numbered 220 to the minute. If the faulty diet is continued a sufficiently long time, the animals die. Whether they die, however, as the result of the loss of rigidity of the thorax or from other causes, we have not tried to determine.

[3] One of the 22 cases is a child just admitted to the hospital. The outcome is uncertain.

[4] Jour. Biol. Chem., 1921, XLV, 333.

DESCRIPTION OF PLATES

FIG. 1.—H. B., aged 17 months. The depression at the side of the thorax is well marked, as is also the rounded ridge created by the posterior angles of those ribs forming the floor of the depression. The rounded elevation formed by the posterior angles of the ribs is the posterior boundary of the depression, and sharply separates the back from the antero-lateral surface of the deformed thorax. The forward inclination of the sternum and the recession of the ensiform cartilage are well shown. The picture of the child is quite typical. Though the legs are flexed, the knees are in contact with the surface of the table.

FIG. 2.—Photograph of E. D., aged 3¼ years. The collapse of the sides of the thorax is extreme. The left side is more sunken than the right as the result of fractures of the ribs.

FIG. 3.—Photograph of S W., aged 3½ years. The deformity of the thorax is typical. The photograph is reproduced to show the high position of the thorax. The manubrium immediately underlies the chin. The neck is marked by a fold. The deformity of the vertebral column is characteristic of rickets. In reality, the child no longer belongs to our series of cases, because the ribs and costochondral junctions had acquired sufficient rigidity through an overproduction of osteoid completely to withstand the forces exerted upon them. The bones of the extremities had become enormously thickened.

FIG. 4.—R. W., aged 28 months. The depressions at the sides of the thorax, when marked, narrow the back. At the level of the scapulæ there is lordosis, but below in the region of the lower dorsal and upper lumbar vertebræ a well marked rachitic kyphosis. The abdomen is large. The child is obliged to support the body with the hands in order to sit. In the most severe cases the child is too weak to sit.

FIG. 5.—Tracings from chest and abdomen of J. R., aged 16 months, to show that the circumference of the thorax may actually undergo a decrease during inspiration. Thin pneumatic bags were placed, one around the middle of the chest so as to cross the lateral depressions, the other around the abdomen. Both bags were covered with thin bands of canvas, filled with air and connected with tambours.

Tracing 1 records the movements of the thorax. Inspiration begins at *a* and ends at *b*. The tracing shows that a progressive diminution in the size of the thorax, measured in the horizontal plane, occurs during inspiration. The sharp rise in the tracing marked *x* was produced as the result of the sudden recoil of the thorax occurring immediately after the inspiratory effort was finished. The thorax seems to snap from the inspiratory to the expiratory position at the end of inspiration like a suddenly released spring.

Tracing 2 shows the increase in the circumference of the abdomen during inspiration. *a* marks the beginning of inspiration, *b* its termination.

The loss of rigidity of the thorax was so great that the slight pressure from the inflation of the pneumatic bags was enough to cause asphyxia and cessation of breathing. The child was resuscitated with great difficulty and the attempt to obtain further tracings abandoned.

It is appreciated that the records obtained by the method described do not give exact measurements of the variations in the size of the thorax during respiration. They show definitely, however, that in severe rickets the thorax may undergo a marked decrease in size at the level of the lateral depressions while inspiration is taking place.

The tracings were obtained through the kindness of Dr. Harold L. Higgins.

FIG. 6.—A section taken in the horizontal plane through the enlargement formed by the junction of the cartilages and rib from the thorax of A. H., aged 14 months. The section was stained with hematoxylin and eosin.

a—shaft.

b—undifferentiated cartilage.

c—cartilage of the proliferative zone.

d—the transitional zone or metaphysis lying between the proliferative zone of cartilage and the shaft. In this zone tongues and bands of cartilage (like the rest of the cartilage appearing black) are interspersed with composites of tissue made up of connective tissue, blood vessels ending in large tufts, degenerated cartilage in the various stages of metaplasia into osteoid, and osteoid itself.

e—connective tissue and osteoid filling in the crevice between the cartilage and transitional zone on the one hand and the outer surface of the shaft on the other.

f—a piece of the visceral pleura which adhered to the pointed end of the shaft protruding through the costochondral enlargements. The visceral pleura rubs against the sharp point of the shaft and finally becomes attached. Although the section shows the tag of visceral pleura, it misses the protruding end of the shaft which lay in another plane than the plane of the section. The cartilage and shaft meet at right angles. The cartilage is joined to the shaft on its outer side.

FIG. 7.—The thorax of W. M., aged 16 months, opened at autopsy. The costal cartilages have been cut through near the costochondral junctions and removed together with the sternum.

a—enlarged costochondral junctions; the cut ends of the cartilages are visible. The cartilages meet the shafts in some instances at right angles. The bulbous character of the enlargements of the costochondral junctions and the knuckle-like projections which they make into the interior of the thorax are well shown. The sharp ends of the shafts of the ribs can be seen protruding through the inner side of the enlargements and forming their summits.

b—grooves on the external surfaces of the lung produced by the pressure of the enlarged costochondral junctions. The thoracic wall has been retracted so that the grooves are exposed to view.

c—emphysematous lung in front of the grooves. As a result of the partial compression of the bronchi by the enlarged costochondral junctions the discharge of air from those portions of the lung internal to the grooves is interfered with. A condition of emphysema is therefore produced.

d—a large part of the left lung is atelectatic.

FIG. 8.—A section taken through the lung of A. H., aged 14 months, to show the combination of atelectasis and emphysema.

a—atelectatic lung underlying the enlarged costochondral junctions.

b—atelectatic lung bordering on the vertebral column.

c—pulmonary tissue in a state of emphysema.

"The left lung is apparently diminished in volume. A vertical groove traverses the lateral aspect of the upper lobe in such a way as to separate the anterior quarter from the posterior three-quarters. The groove is formed by atelectatic tissue. In front of the groove the lung is downy and yellow, in a state of emphysema. Behind the groove the lung is pink, showing areas of emphysema and some atelectasis. The groove traverses also the lingula, separating the tip which forms a rounded inflated mass by a neck of atelectatic tissue from the portion behind. The paravertebral parts of both lower and upper lobes are in a state of atelectasis. The surface is depressed and dark blue and the interlobular tissue is visible. Scattered elsewhere through the lower lobe are bluish depressed areas of atelectasis."

"The right lung is also reduced in bulk. The groove marked by the atelectasis is even more conspicuous. Where it crosses the middle lobe it is 2 cm. in width and the floor thereof 2 mm. in thickness. That part of the middle lobe which lies in front of it is distended to its utmost capacity and is downy and white. The grooving of the upper lobe is very pronounced. Fully one-half of this lung appears collapsed. All that part in the paravertebral groove and by far the greater part of the rest of the lower lobe is atelectatic. The apex and that part of the lower lobe which lies in front of the groove above mentioned are extremely distended. Viewing the lung from the inner side and below, it is seen that the atelectasis occupies large areas, tending to be most marked in the region of the hilus—but extending to the periphery." [Quotation from autopsy protocol.]

FIG. 9.—Views of the normal thorax; *a*—cut across at the level of the sixth rib and viewed from below; *b*—viewed from in front.

FIG. 10.—Views of the deformed thorax in rickets.

a—a not very severely deformed thorax cut across through the sixth rib and cartilage, viewed from below. Note the asymmetry. The projections inward of the deformed costochondral junctions divide the thorax into two chambers. In the anterior one the heart is contained. As usual the recession inward of the thoracic wall is greater on the right side than on the left, and the arch of the ribs and cartilages correspondingly increased. Attention should be called to the fact that the thorax is in the position in which the deformities are least marked. If the drawing had been made from the thorax in the position of inspiration instead of expiration, the degree of deformity would have been increased almost two-fold. The costochondral junctions would have been 1 cm. or more nearer the vertebral column; the anterior chamber would have been correspondingly narrowed and the arching of the ribs increased.

b—The thorax of the same child, viewed from in front. The malformation is more marked on the right side than on the left. The enlargement of the costochondral junctions is very slight externally but well marked on the inner surface of the thorax. Owing to the collapse of the thoracic wall inwards and backwards toward the vertebral column, the costal margins have been drawn upwards and backwards over the surface of the liver, so that a much larger part of the latter is uncovered than under normal conditions.

c—the junction of cartilage and shaft of the rib viewed from in front. The cartilage has been twisted about probably as the result of the rotatory movements to which the shaft of the rib is subject, so that it no longer lies in line with the shaft even in the horizontal plane. The enlargement is much greater on the inner than on the outer side.

d—junction of cartilage and shaft seen from below. The projection of the sharp end of the shaft through the enlargement formed by the abnormal junction of cartilage and shaft is well shown. The projecting point of the rib forms the summit of the enlargement.

FIG. 11.—Photograph of the heart of A. H., aged 14 months.

a—left ventricle;

b—right ventricle. Note the hypertrophy of the internal musculature of the right ventricle.

"Right chamber shows great hypertrophy and is dilated. Its wall measures 3 to 5 mm. in thickness. The wall of the left ventricle close to the aortic ring measures 1 cm. The internal musculature of the left ventricle seems normal. The internal musculature of the right ventricle is hypertrophied. Valves normal. Weight of heart 85 gm." [Quotation from autopsy protocol.] (Body weight not recorded.)

FIG. 12.—X-ray photograph of chest of C. H., aged 10 months. The ribs appear extremely slender and porous. They do not show the normal inclination and parallel arrangement. Some of them are greatly bent. On either side of the shadow cast by the heart are the longitudinally directed shadows cast by the strips of atelectatic lung underneath the enlarged costochondral junctions. The longitudinal shadow on the right side is separated from the shadow of the right border of the heart by a just perceptible interval. The shadow on the left side skirts the left border of the heart above and merges with it below. Evidently the heart extends from one row of costochondral junctions to the other. The lungs beyond the longitudinal shadows have a hazy appearance. The evidences of rickets in the humeri and scapulæ are well marked. The X-ray photograph is typical.

FIG. 13.—The X-ray photograph of thorax of V. F., aged 17 months. The heart shadow is well defined and large in comparison to the size of the thorax. On the right side of the heart shadow and on the left side are shadows caused by the deformed chest wall. The sides of the chest have sunken to such an extent that the portions of the thoracic wall forming the anterior parts of the depressions lie in the antero-posterior plane, that is, parallel to the Roentgen rays in-

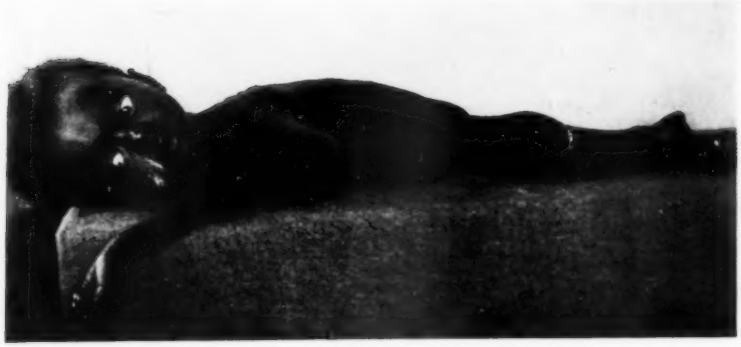

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

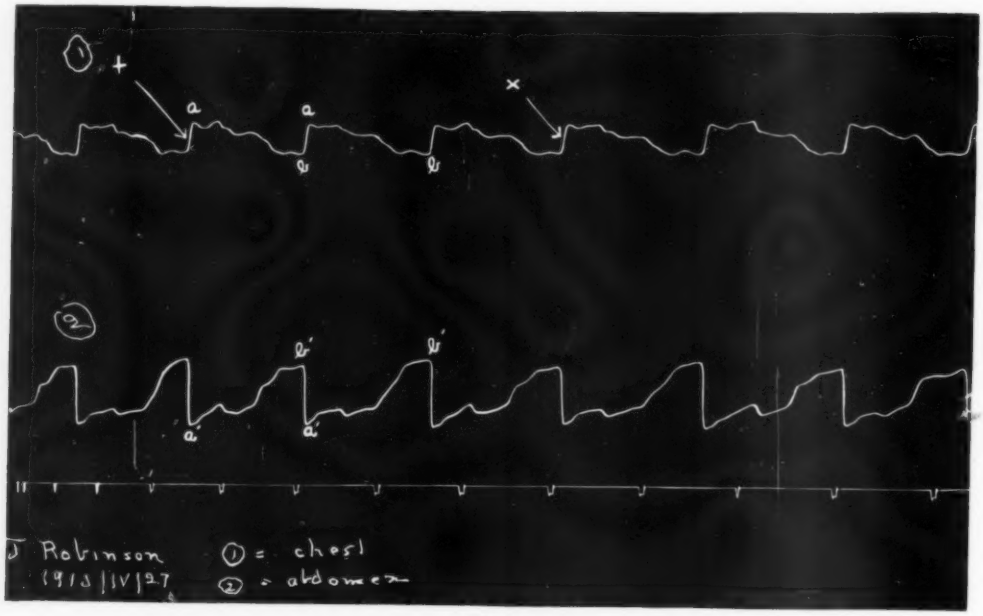

FIG. 5.

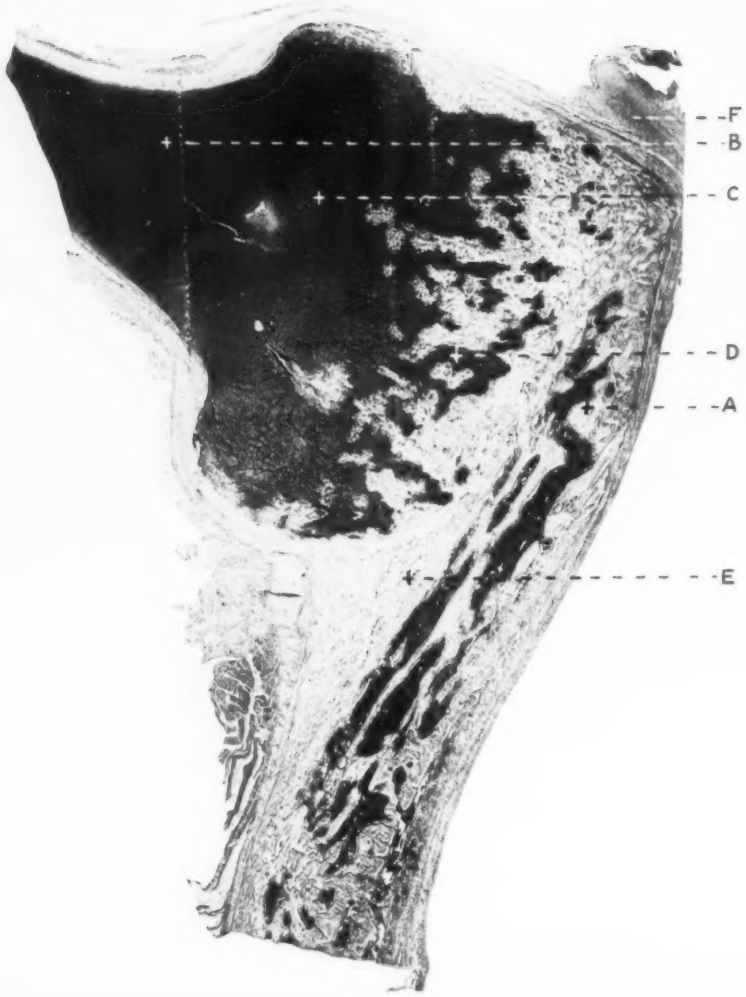

FIG. 6.

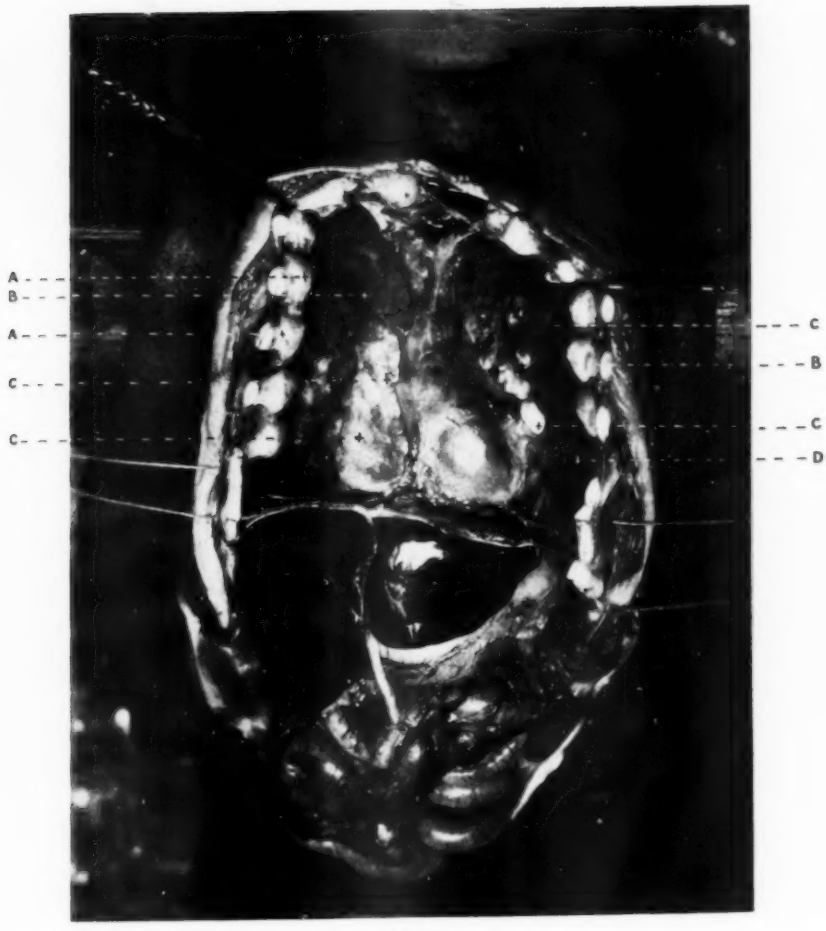

FIG. 7.

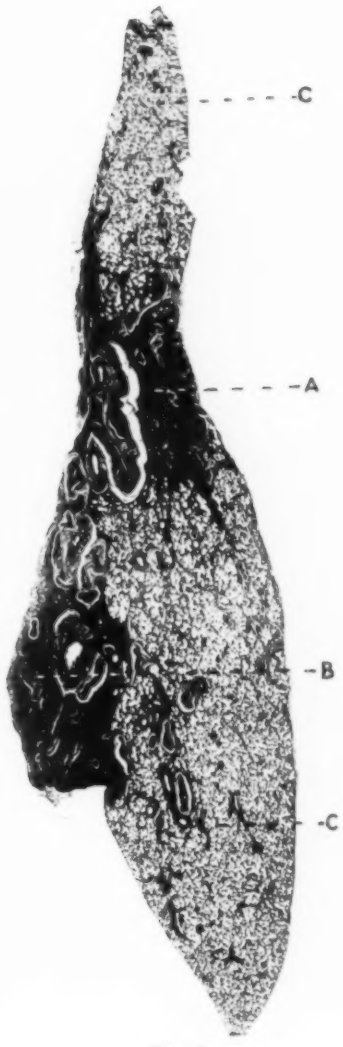

FIG. 8.

FIG. 9.

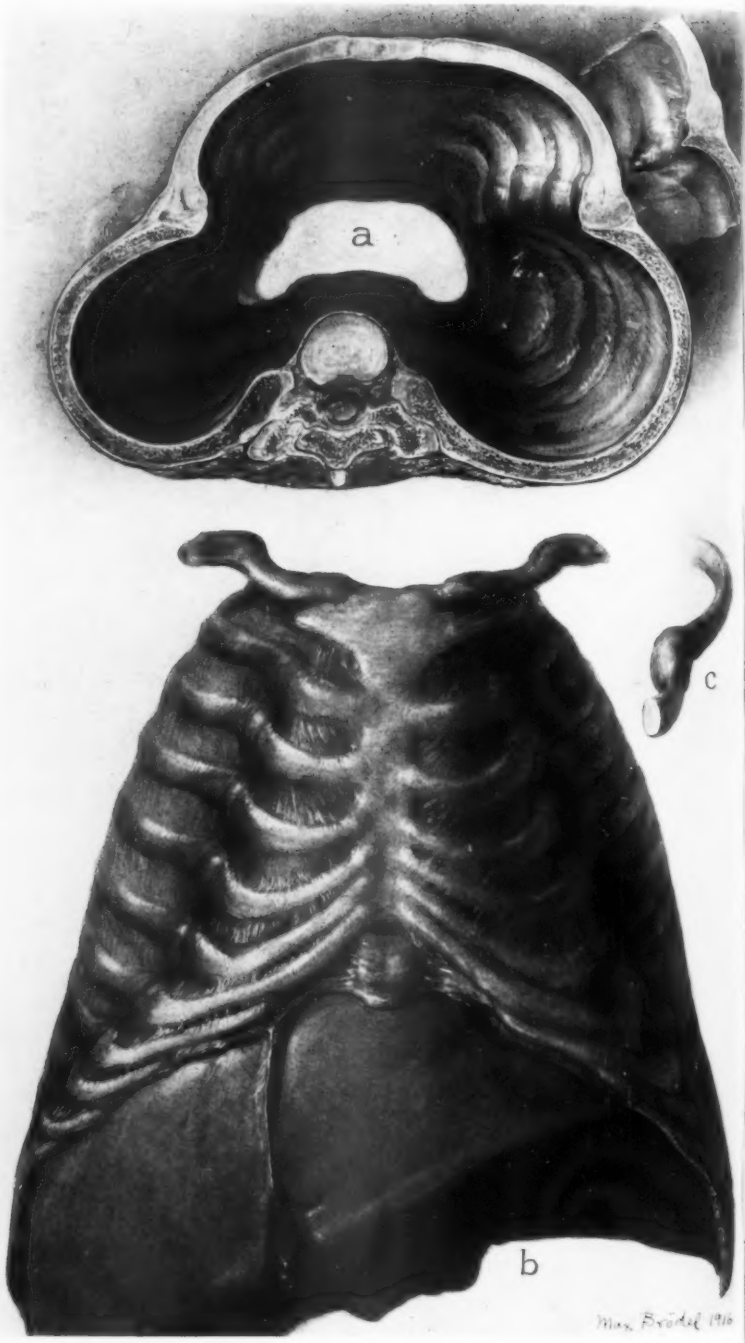

FIG. 10.

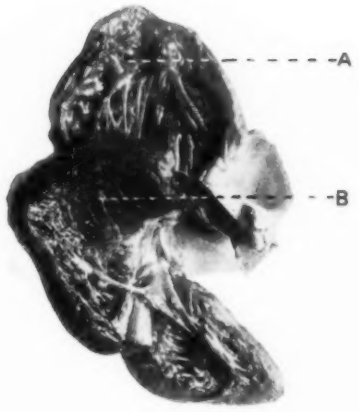

FIG. 11.

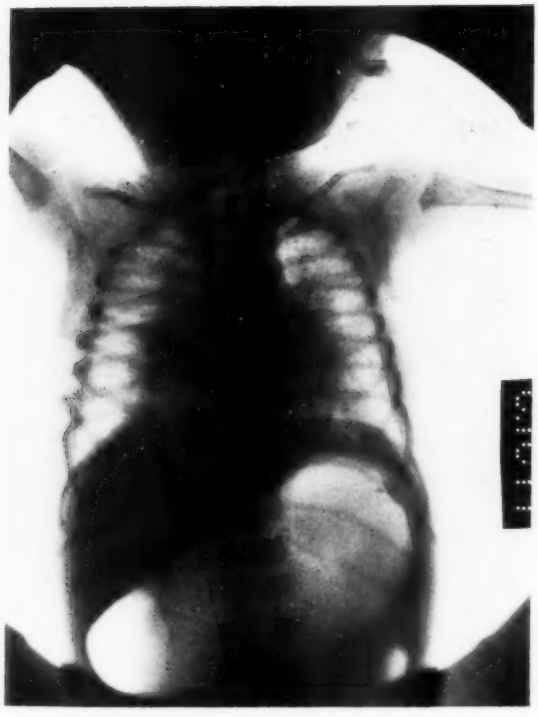

FIG. 12.

FIG. 13.

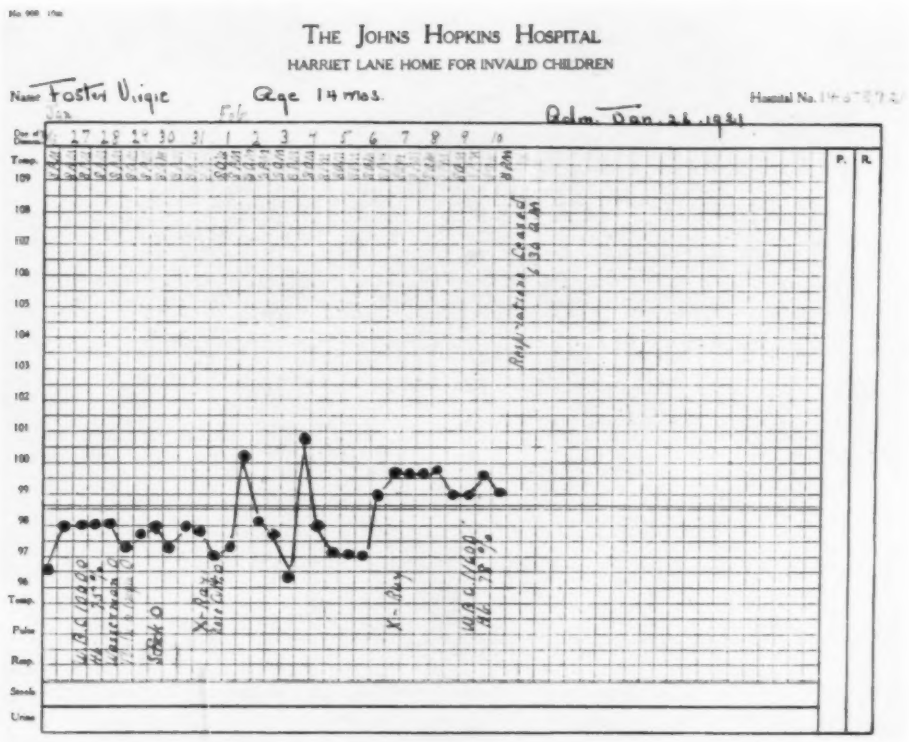
THE JOHNS HOPKINS HOSPITAL
HARRIET LANE HOME FOR INVALID CHILDREN
Name Foster Uhrie
Age 14 mos.
Adm. Jan. 26, 1921
Temp.
Pulse
Resp.
Stools
Urine
P.
R.

FIG. 14.

FIG. 15.

stead of perpendicular to them. The shadow on the right side completely outlines the anterior margin of the depression. The shadow on the right side cast by the atelectatic lung underneath the enlarged costochondral junctions is merged with the shadow cast by the deformed chest wall while on the left side the shadow is continuous with the shadow cast by the latter. The heart must touch the row of costochondral junctions on the left side and extend almost to the row on the right. The ribs have a porous appearance and are abnormally bent. Scoliosis of the vertebral column is present.

The X-ray photograph in this case, showing so plainly the deformity of the chest wall, is unusual. It is reproduced for that reason.

FIG. 14.—Temperature chart of V. F., aged 17 months. Throughout the period of observation in the hospital the respirations during waking or sleep were never less than 60 to the minute. The thorax was greatly deformed and weakened. With each inspiration the collapse of the sides was extreme. The physical signs in the lungs were characteristic. There was no evidence of a pneumonic process either on physical examination or on examination with the X-ray. The respirations became more and more labored. Death seemed to occur from exhaustion.

FIG. 15.—A segment of the thorax of a rachitic rat viewed from below. Note that the costochondral junctions form marked prominences internally and divide the thorax into two chambers. In the anterior chamber the heart is, so to speak, imprisoned, exactly as in the case of the human subject. Note the abnormal curvature of the shafts of the ribs, and the asymmetry.

DIPHTHERIA BACILLUS CARRIERS

A REPORT OF CONDITIONS FOUND IN AN ORPHAN ASYLUM

By W. L. Moss, C. G. GUTHRIE and J. GELIEN

(From the Division of Clinical Pathology of the Medical Clinic, The Johns Hopkins University and Hospital)

During an investigation of diphtheria bacillus carriers in the winter and spring of 1912 * throat cultures were taken from 800 children attending one of the public schools of Baltimore. From the carriers disclosed by the examination of these cultures, 50 were selected at random for further study. Throat cultures were taken from this group of 50 carriers at intervals of two weeks for ten weeks and a fortnight later cultures were taken from the entire 800 children. There were thus seven observations on 50 of the children and 2 on the remaining 750 over a period of three months. At the time of each of these seven observations, the condition of the throat was noted and the temperature was taken at each examination except the first. An attempt was made to isolate diphtheria bacilli from each of the positive cultures encountered, and the strains isolated were then tested tinctorially, culturally and for virulence. Some weeks after the last series of cultures, a systematic effort was made to determine the relation of the carriers found to the community at large. The detailed results of this investigation may be found on reference to the paper cited above but are summarized briefly here.

1. The first examination in February, 1912, revealed 85 carriers among the 800 children, or 10.62 per cent. Re-examination of the same 800 children three months later showed 69 carriers, or 8.62 per cent. It was a striking fact, however, that only 10 of the children gave positive cultures at both examinations. The number of children yielding positive cultures at either one or the other examination was 144, or 18 per cent.

2. Definite statements could not be made with regard to the actual duration of the carrier state in the 50 carriers selected for repeated study, but our findings indicated a progressive diminution in the number of positive cultures found at the successive examinations and at the last examination only six showed diphtheria organisms.

3. The diphtheria bacilli found in the throats of these carriers were typical Klebs-Loeffler organisms, but many of the strains were non-virulent. The non-virulent strains apparently differed from the virulent ones only in their ability to produce toxin. Since the other members of the diphtheria group encountered in the throat were readily distinguished from *B. diphtheriæ*, they caused very little difficulty in diagnosis and were considered of no pathogenic importance.

4. Virulence tests showed that only about 11 or 12 per cent of the carriers which we studied harbored virulent organisms and that of the total positive cultures obtained only about 10 or 11 per cent were virulent.

5. No convincing proof of a change in virulence of the organisms in the throat was obtained.

6. To quote directly concerning the relation existing between the presence of diphtheria bacilli and pathological throat conditions other than clinical diphtheria: "Our general impression upon this point is that the mere presence of diphtheria bacilli in the throats of carriers is not responsible for any objective pathological condition whatever, and does not cause an elevation of temperature. On the other hand, persons with pathological throat conditions other than clinical diphtheria, particularly those with diseased tonsils, seem to furnish a somewhat more favorable field for the lodgment and growth of the diphtheria bacillus than that afforded by persons with normal throats."

7. Regarding the relation of the 160 carriers investigated by us to the occurrence of clinical diphtheria either in themselves or in their associates:

(a) Only 11 gave a history of having had diphtheria, but none more recently than three years previous to the time of our study.

* Guthrie, C. G., Gelien, J., and Moss, W. L.: Diphtheria Bacillus Carriers: Second Communication, Johns Hopkins Hosp. Bull., 1920, XXXI, 388.

(b) None of the carriers subsequently developed diphtheria during the period that they were under observation.

(c) Fourteen gave a history of exposure to diphtheria at a period varying from 1 to 12 years previous to the time cultures were made.

(d) A painstaking investigation revealed no case of diphtheria among any of the associates of the 160 carriers.

As a result of these findings we felt justified in emphasizing certain points as follows:

1. The wide prevalence of diphtheria bacillus carriers.

2. The great preponderance of non-virulent bacillus carriers over virulent bacillus carriers.

3. The inability of non-virulent diphtheria bacilli to produce diphtheria.

4. The harmless nature of non-virulent bacillus carriers.

5. The injustice of interfering with the liberties of carriers of non-virulent diphtheria bacilli by quarantine or other measures.

6. The erroneous nature of information concerning the part played by carriers in the spread of diphtheria unless consideration is given to the nature of the organisms harbored by them.

Certain additional information obtained from our study of these children but not embodied in the original report, illustrated in a striking manner one phase of the diphtheria carrier problem, and forms the basis of the present paper.

On looking over the list of 85 carriers detected in the first 800 cultures, some very interesting facts were noted. First, a very large number of these carriers—26, or 30.59 per cent—came from the same street address; second, among the 50 carriers selected at random for repeated examination, 22, or 44 per cent, came from this address; third, among the 81 children from this address, who were examined, 32.09 per cent gave positive cultures. This state of affairs seemed to demand further investigation, which showed that the street number in question was that of an orphan asylum. The information obtained concerning this institution from the superintendent who has had charge of the asylum since 1894 was as follows: The asylum was founded in 1863 and has occupied its present location since 1874. White children only are admitted, but no distinction is made as to nationality or religion. Beyond this the only requirements are (1) that the child is an orphan, (2) is from 3 to 10 years of age, (3) is pronounced sound by the regular medical examiner of the institution and (4) that the financial condition is such as to require aid. At the time our cultures were taken there were 123 inmates, exclusive of the executive staff and attendants, 57 boys between the ages of three and 13 years, and 66 girls ranging in age from three to 17 years. Eighty-five, or 69.17 per cent, of these 123 children attended the public school in which our cultures were taken and 81 of them were included in the first examination of our series and so came under subsequent observation. Of these 81 children, 45 were boys from five to 13 years of age and 36 were girls from six to 14 years of age. Twenty-two of them chanced to be among the 50 carriers selected for repeated examination and thus were recultured at intervals of two weeks over a period of three months. At the final examination practically all of the original 800 children were recultured. Seventy-nine of the 81 children previously studied from the asylum had cultures taken at this time. As was mentioned before, observations on the condition of the throat were also made at each examination and the temperature taken at each examination except the first. The detailed results obtained have been analyzed and are here presented briefly, followed by such comment as seems warranted.

1. *Incidence of Positive Cultures.*—A total of 270 cultures was taken from the 81 inmates of the asylum and 56 positive cultures were obtained; in other words, 20.70 per cent of the cultures taken showed diphtheria bacilli.

Cultures positive at 1st examination of 81 children = 26 (32.09 per cent)
" " " 2d " " 22 " = 3 (59.09 " ")
" " " 3d " " 22 " = 7 (31.81 " ")
" " " 4th " " 22 " = 1 (4.54 " ")
" " " 5th " " 22 " = 3 (13.63 " ")
" " " 6th " " 22 " = 2 (9.09 " ")
" " " 7th " " 79 " = 4 (5.06 " ")

Total cultures 270. Positive 56 (20.70 per cent)

Thirty, or 37 per cent, of the 81 children, at some time showed a positive culture. A total of 168 cultures was taken from these 30 individuals and of these cultures 56, or 33.21 per cent, were positive.

Individuals	Times cultured	Total cultures	Positive cultures
1	1	1	1
7	2	14	7
1	6	6	3
21	7	147	45
30		168	56

Of the 21 carriers cultured seven times, 8 were positive at only the first examination; 8 were positive twice; 1, three times; 3, four times; 1, six times; and none showed a positive culture at all of the seven examinations.

We failed to find diphtheria organisms in the cultures from 51 of the 81 inmates of the asylum examined but it should be pointed out that each of these 51 children had only two examinations, three months apart.

We were unable to discover any relation between the age or sex of the children and the occurrence of positive cultures.

2. *Character of the Organisms Present in the Positive Cultures.*—This has been dealt with at some length in our earlier report to which reference has been made. In this place it will be sufficient to say that organisms were isolated from 48 of the 56 positive cultures encountered, and these pure strains examined as to morphology, staining characteristics and cultural reactions. The results showed these strains to be typical diphtheria bacilli, not "pseudo-diphtheria bacilli," nor diphtheroids.

Cultures from 26 of the 30 carriers were tested for virulence. From a number of these children who repeatedly showed positive cultures, pure strains were isolated from the successive

positive cultures and virulence tests made upon each of the pure strains, as follows:

1	strain	tested	from	each	of	15	carriers	= 15	strains
2	strains	"	"	"	"	7	"	= 14	"
3	"	"	"	"	"	2	"	= 6	"
4	"	"	"			1	carrier	= 4	"
5	"	"	"			1	"	= 5	"
						26	carriers	44	strains

Unfortunately four strains which had been isolated were lost before this test could be made and from 8 of the positive cultures no isolation was made. We are thus unable to say whether the strains of diphtheria bacilli in these 12 cultures were toxin producers or not, and mere speculation on this point is unprofitable. Concerning the 44 strains from 26 carriers which were tested, however, there is no doubt; all of these strains were avirulent.

3. *Occurrence of Pathological Throat Conditions.*—As has been mentioned, the throats of these children were examined and the occurrence of outspoken pathological conditions such as pharyngitis and enlarged or diseased tonsils was noted each time that cultures were taken. Of the 81 children from the asylum who were included in our study, 35, or 43.20 per cent, at some time showed a throat condition regarded as definitely pathological. Of these 35 children who at some time exhibited a diseased condition of the throat, fifteen were examined twice, one was examined six times, and 19 were examined 7 times, making in all 169 examinations. At the 169 examinations on the 35 children, a pathological condition of the throat was noted 78 times, as follows: 17 children showed it but once; 6 showed it twice; 5, three times; 3, four times; 3, five times; and one individual showed it on seven successive examinations. Of the 81 children studied, 46 or 56.79 per cent did not show a pathological throat condition but it should be pointed out that only two of these individuals were examined 7 times; 43 of them had but 2 examinations and one child was examined only once, thus making 101 examinations on these 46 children as contrasted with 169 examinations on the 35 children who at some time showed pathological throat conditions. It is quite probable that more frequent examinations would have materially increased the number of children discovered to have diseased throats. It should be stated that in no instance was a diphtheritic false membrane or pathological membrane formation of any sort observed.

4. *Relation of Pathological Throat Conditions to the Carrier State.*—This matter also has been considered in the earlier report and the impression which we have gained has been expressed in the *résumé* at the beginning of this paper. This impression has not been modified by the findings in this particular group of children. The occurrence of pathological throat conditions and their association with positive throat cultures is presented in this place for a reason which will later be apparent.

The number of children in whom positive cultures and pathological throats were found at the same examination was 14, which is 46.66 per cent of the 30 individuals with positive cultures and 40 per cent of the 35 individuals with pathological throats. Ninety-two cultures were made from these 14 children and of these 35, or 36.95 per cent, were positive. Of these 35 positive cultures, 23, or 65.71 per cent, were obtained from throats noted as pathological at the time the culture was taken. Of the 35 children who at some time showed a pathological throat 22, or 62.85 per cent, at some time showed a positive culture. From these 35 children 169 cultures were made and 45, or 26.62 per cent, proved to be positive. As mentioned above, 22 children who at some time showed pathological throats, at some time were found to be harboring diphtheria bacilli; from these children 143 cultures were taken and 45, or 31.46 per cent of the cultures were positive.

Positive cultures were found in only 8 of the 46 children who at no examination showed a pathological throat condition. From these 8 children 25 cultures were taken and 11, or 44 per cent of the cultures, were positive. In all, 101 cultures were taken from children not noted as having diseased throats and 11, or 10.89 per cent, were positive.

Thirteen of the 35 children with pathological throats had negative cultures, 37.14 per cent. From these 13 children 26 cultures were taken.

Thirty-eight, or 44.44 per cent of the total of 81 children examined from the institution, showed neither pathological throat nor positive culture. From these 38 children 76 cultures were taken.

5. *Temperature Observations.*—Many of the children had an elevation of temperature above 99° F. and in one child it was 100.6° F. although all were attending school at the time our observations were made. These elevations occurred in children showing positive cultures and in those whose cultures were negative; in children with pathological throats and in those whose throats seemed normal; and in children having the various combinations of cultural result and condition of the throat. We did not gain the impression that the carrier state was in itself sufficient to cause an elevation of temperature, although not infrequently associated with such elevations. The condition of the throat noted in many of the children would offer a plausible explanation for an elevation of temperature, but the slight fever present in those with normal throats is not to be explained in this way, nor is it necessary for our purpose to search for the underlying cause in these cases. It is merely desired to emphasize the point that many of the children with positive cultures had also elevations of temperature and pathological throat conditions.

7. *Inmates of the Same Institution Examined the Previous Year (1911).*—During an investigation of diphtheria bacillus carriers in the previous year (1911) *, cultures were taken from 200 children attending the same public school from which the material for our later report was secured. Included among these 200 children were 17 from the orphan asylum in question, about 14 per cent of the inmates at that time. Twenty-one cultures were made from the 17 children and only 1 was

* Moss, W. L., Guthrie, C. G., and Gelien, J.: Diphtheria Bacillus Carriers, Trans. XV Internat. Cong. on Hyg. and Dem., 1912, IV, 156.

found positive. In the course of the present inquiry, 8 of the same children who had been examined during the previous year came under observation and the number of cultures taken from them was 21. None of these 8 children had shown a positive culture in 1911, but 2 of them proved to be carriers when examined in 1912.

8. *Relation of the Carriers to the Occurrence of Clinical Diphtheria.*—Subsequent investigation of the 30 carriers from this institution found in the course of our study of 800 children from one of the Public Schools of Baltimore showed that a history of diphtheria previous to the time of our examination could be obtained from only three individuals. Concerning one child a history was obtained of diphtheria "when a baby"; regarding another, a history of diphtheria "when a small child"; the third had had diphtheria five years before the time that our cultures were taken. In each of these three instances, the disease had occurred before the child became an inmate of the asylum.

None of the 30 carriers developed diphtheria during the period covered by our study, from three to four months. None gave a history of previous exposure to diphtheria. We were unable to discover any cases of clinical diphtheria developing among the associates of these carriers. Six of the 30 carriers gave a history of sore throat during the year, but all had recovered without the aid of a physician.

Perhaps the most interesting information with regard to the relation of these carriers to the community was obtained from the superintendent of the asylum, who was interrogated in 1914 and again in November, 1920. This man has been in charge of the institution since 1894 and his records show that during the 26 years of his incumbency there have been only five cases of diphtheria among the inmates of the asylum. These five cases had occurred in the form of a small outbreak in 1897, 15 years before the time of our investigation. No cases had occurred since that time; on this point he was quite definite.

The question naturally arises as to why we did not investigate the remaining 42 inmates of this interesting orphan asylum and it must be confessed that the explanation, which then seemed adequate, now appears distinctly feeble. Although we had the sanction of the Health Department for our investigations in the public schools we had no authority to invade private institutions. The superintendent of the orphanage, who regarded us with open suspicion, did not take kindly to the idea when casually approached on this subject and no pressure was brought to bear on him, as we were particularly anxious not to call attention to the carrier situation existing in the asylum at that time. Our discovery of this situation was merely incidental in the course of a rather extensive investigation of carriers in which we were engaged and which occupied us fully, so the matter was not pushed.

Summary.—The high incidence of *B. diphtheriæ* in the throats of children from this institution was very striking, as 37 per cent of the 81 inmates examined at some time showed positive cultures, and 20.7 per cent of the total 270 cultures taken contained diphtheria bacilli. There is no reason to suppose that the incidence differed among the other inmates of the asylum, but if the 42 children not examined had all shown negative cultures, there would still have been 24.39 per cent of carriers in the entire institution, a remarkably high figure. Owing to conditions not understood, the ordinary percentage of carriers was in this instance greatly exceeded; this, in turn, served to increase markedly the percentage of carriers found among 800 children from the public school attended by the inmates of this asylum. The incidence of carriers was also much higher than that revealed by the examination of 21 cultures from 17 inmates of the asylum during the previous year, when only one child was found to have diphtheria bacilli in the throat. During the period covered by our observations there was a marked fall in the incidence of carriers; at the first examination 26, or 32.09 per cent of 81 children, had positive cultures; three months later only 4, or 5.06 per cent of the 79 children examined, were found to be harboring diphtheria bacilli in their throats. There was a progressive decrease also in the number of positive cultures obtained at successive examinations of the 22 carriers included by chance in the list selected for repeated study (22, 13, 7, 1, 3, 2, 0). No relation could be discovered between the age or sex of the children and the occurrence of positive cultures.

The identity of the organisms found in the throats was not a matter of surmise, as diphtheria bacilli were isolated from 48 of the 56 positive cultures encountered and these pure strains subjected to careful tinctorial and cultural tests. All proved to be typical in their morphology, staining characteristics and cultural reactions.

Virulence tests were carried out on 44 of the 56 positive cultures encountered, representing pure strains from 26 of the 30 carriers found in this institution. All of the strains were non-virulent.

Many of the children (43.2 per cent) showed throats which were regarded as definitely pathological in appearance and of these children 62.85 per cent had positive cultures. Among the children whose throats seemed normal, only 17.39 per cent had positive cultures. The percentage of positive cultures was also higher among the children with pathological throats (26.62 per cent) than among those with normal throats (10.89 per cent). None of the throats examined showed a diphtheritic membrane or pathological membrane formation of any sort.

Temperature elevations above 99° F. were frequently noted among the inmates of the asylum examined. Such elevations occurred among the children with normal-looking throats whose cultures were positive and among those whose cultures were negative; they occurred in children with evidently pathological throats who showed negative cultures, but the point of interest to us was that many of the carriers with diseased throats also showed a slight fever.

Only 3 of the 30 carriers discovered among the inmates of the institution had previously had diphtheria, none less than five years before the date of our examination, and none since admission to the asylum. None of the children gave a history of previous exposure to diphtheria. A thorough investigation did

not reveal a single case of diphtheria developing among any of the associates of these carriers. There had not been a case of diphtheria in the asylum for 15 years prior to our study; there has not been a case in the eight years which have elapsed since that time.

Extensive comment on these findings seems unnecessary; it will suffice to correlate some of the points brought out by our study and indicate their practical bearing on the problem of diphtheria bacillus carriers. It has been shown that 30 diphtheria bacillus carriers were found on examination of only two-thirds of inmates of the asylum; that pathological throat conditions were common and were frequently found among the diphtheria carriers; that slight fever was also common among the children and occurred among the bacillus carriers having pathological throat conditions. The discovery of a positive culture in a child with an evidently pathological throat and an elevation of temperature might readily suggest the diagnosis of diphtheria and, indeed, many diagnoses are made on no more evidence than this. It should be recalled, however, that in none of these pathological throats was there observed a false membrane or new membrane formation of any sort. Moreover, the 44 cultures tested were all avirulent and therefore presumably incapable of producing any lesion whatever, much less clinical diphtheria. Finally and most important, there is the practical result. No one but ourselves knew of the carriers in the orphanage, consequently the lives of these children were not interfered with in any way and the daily routine of the institution remained undisturbed. The carriers themselves did not develop diphtheria; so far as we could learn their associates did not develop diphtheria; to the best of our knowledge there has been no diphtheria in the asylum for 23 years.

Nevertheless, the possibilities of trouble for the institution at this time were tremendous. Had a case of diphtheria occurred in the public school attended by these children some of them might have had throat cultures taken as contact suspects, and this in turn might have led to investigation of the orphanage. There the apparently alarming state of affairs outlined above would have been discovered, at least in part. Many of these harmless carriers of non-virulent organisms would probably have been regarded as cases of diphtheria, on the basis of a pathological throat, an elevation of temperature and a positive culture. The establishment of a flourishing pseudo-epidemic only awaited the arrival of some well-meaning enthusiast. The institution would have been placarded at once, the children excluded from school, and many of them needlessly subjected to the discomfort of injections of antitoxin for curative or prophylactic purposes. At the end of 12 weeks, as we have seen, the incidence of positive cultures had dropped from 32.09 per cent to 5.06 per cent; this might plausibly have been attributed to the antitoxin used and the measures employed for isolation of the patients. All of these things might very readily have occurred in this orphan asylum; similar occurrences probably take place very frequently.

CONCLUSIONS

1. The carrier of avirulent diphtheria bacilli is not a menace to the community.

2. A positive throat culture, an elevation of temperature and a pathological throat condition without definite membrane formation are insufficient evidence on which to base a diagnosis of diphtheria with entire certainty.

3. Virulence tests are necessary to avoid inflicting needless hardships on carriers of avirulent diphtheria bacilli.

STUDIES WITH LECITHIN AND CHOLESTERIN IN RELATION TO THE ANTIHEMOLYTIC PROPERTY OF HUMAN SERUM

By HALLIE M. CLARK and FRANK A. EVANS

(From the Division of Clinical Pathology of the Medical Clinic, The Johns Hopkins University and Hospital)

It has been well established that human serum has power to inhibit hemolysis by many different agents,[1] among others soluble soaps and fatty acids, substances occurring in the blood under normal conditions. It is probable that because of this power of the serum the hemolytic substances present in the blood do not cause destruction of the red blood cells in the body. This consideration is of interest since it has been shown [2] that the serum from patients with anemia, as compared with that from normal persons and patients with no anemia, exhibits a diminution in protective power against hemolysis of guinea-pig corpuscles by sodium oleate. The diminution in the protective power of the serum against this hemolytic agent is much more pronounced, both in degree and the regularity with which it is found, in the so-called hemolytic anemias, Addisonian anemia and hemolytic icterus, and in conditions in which the liver or spleen is extensively or prominently involved in the disease process. The experiments from which this observation was drawn, although affording no proof for the following suggestion, at least raised the possibility that the hemolysis in the hemolytic anemias may be due to hemolytic agents normally in the blood, enabled to act as hemolysins because of the diminution in the property of the serum which normally inhibits such activity. For these reasons it appeared important to study closely the substance or substances in the serum responsible for the protective power exhibited by serum against hemolysis.

Among the substances in the serum that might be considered in some way related to hemolysis or the inhibition of hemolysis, lecithin and cholesterin were selected for study first. In studies of hemolysis much attention has been given to the activity of both lecithin and cholesterin and it has been shown that they are of great importance in this connection.

The facts so far demonstrated, however, do not permit one to postulate clearly just what their action in relation to hemolysis may be in the body. Thus, lecithin itself is a hemolytic agent, and hemolysis by lecithin may be inhibited by cholesterin. Cobra venom is activated by lecithin with the production of a new substance in itself hemolytic, the so-called cobra-lecithid. This cobra-lecithid hemolysis is inhibited by cholesterin, the inhibitory action being due to the interaction of lecithin and cholesterin, possibly the solubility of one in the other.[3] Blood serum may activate cobra venom as lecithin does, and it may also inhibit the activity of cobra-lecithid as cholesterin may do. These properties of blood serum probably depend upon its content of lecithin and cholesterin. In studies with the Much-Holtzmann Psychoreaction, Bauer[4] suggests that increases in the protective power of serum against cobra venom hemolysis probably are due to the paucity of activating lipoids, perhaps lecithin, rather than to an increase in inhibiting substances such as cholesterin. In contradistinction to its action in cobra venom hemolysis where lecithin acts as an activator, with some hemolytic agents it acts rather as a protective substance and tends to inhibit hemolysis caused by them. Thus Arrhenius[5] reported that, if red blood cells are exposed to a lecithin solution beforehand, their resistance to hemolysis by saponin is markedly increased. This same investigator made the observation also that red blood cells so treated exhibit a decrease in resistance to hemolysis by acids, but no alteration in resistance to hemolysis by alkalies. In addition to this, Bayer[6] states that lecithin protects slightly against hemolysis by bile salts. Cholesterin, as compared with lecithin, is not in itself a hemolytic agent and has not as yet been shown to combine with another substance to form an active hemolysin such as occurs with lecithin and cobra venom. Cholesterin does, however, inhibit hemolysis by lecithin and cobra-lecithid. Ransom[7] has shown that cholesterin prevents the hemolysis caused by saponin. It is interesting that lecithin also inhibits hemolysis by this substance;[3] but hemolysis by another substance, bile salts, which is retarded by lecithin, is not influenced by cholesterin.[6] According to Liebermann and Fenyvessy,[8] if cholesterin is boiled with sodium oleate, the hemolytic power of the sodium oleate will be destroyed. In this instance another kind of interaction between lecithin, cholesterin and hemolytic agents is presented, namely, hemolysis inhibited by cholesterin but not by lecithin, for, as brought out in some of the experiments to be reported below, lecithin will not inhibit hemolysis by sodium oleate. From this brief discussion it will be seen that several kinds of relation between lecithin, cholesterin and hemolytic agents have been demonstrated. Thus lecithin is itself a hemolytic agent, and hemolysis by it is inhibited by cholesterin. Lecithin combines with cobra venom to form an active hemolysin, the activity of which is prevented by cholesterin. Lecithin inhibits hemolysis by several substances, hemolysis by one of which, saponin, is also inhibited by cholesterin; but with another, bile salts, no inhibitory power is exhibited by cholesterin. And cholesterin may, under certain conditions, inhibit the action of sodium oleate against which lecithin is inert as a protective agent.

In view of the points discussed above it is of interest that Bloor and Knudson[9] reported a hypocholesterinemia in pernicious anemia, and Gorham and Myers[10] in all anemias of pernicious type and in cachexias of malignancy. Of equal interest is the observation of Denis[11] that after splenectomy in one case of pernicious anemia, one of family jaundice, one of "splenic anemia," and one of Banti's disease, both the lecithin and cholesterin were increased in the whole blood and plasma, and that with improvement in the blood picture and clinical condition the cholesterin percentage rose.

Thus, although the rôle of lecithin and cholesterin in any hemolytic process which may occur in the body may not be stated definitely, there are several facts established which, when considered together, are suggestive as regards the protective property of the serum which may be of importance in relation to anemia and point to these substances for study in this connection. These facts are: first, the diminution in the protective power of serum from cases of so-called hemolytic anemias against hemolysis of guinea-pig corpuscles by sodium oleate; second, the protective power manifested by cholesterin against the action of some hemolysins; and third, the diminution of blood cholesterin in anemias of the pernicious type. If any close interrelation between these facts could be demonstrated it might be possible to say with some assurance upon what substance the antihemolytic property of blood serum depends. More particularly, light might be shed on the nature of the antihemolytic substance in the serum which may be of importance in hemolytic anemias. Accordingly, the following experiments were undertaken to correlate, if possible, the lowered cholesterin in the blood and the diminished protective power of the serum against hemolysis by sodium oleate in the so-called hemolytic anemias. Because of the intimate relationship existing between lecithin and cholesterin in the body and the complexity of their action in hemolytic processes, it seemed probable that if one of them were concerned in any hemolytic process the other would be involved also, and for this reason parallel studies were carried out with lecithin. As a control observation all experiments were done not only with sodium oleate, a hemolytic agent probably occurring normally in the body, but also with saponin which is a hemolysin foreign to the body tissues.

The experiments undertaken to study the points outlined above were as follows:

(1) Quantitative measurements were made of the protective power against hemolysis by sodium oleate and saponin of serum extracted with petroleum ether, and of the petroleum ether extract of serum.

(2) The activities in relation to hemolysis of lecithin and cholesterin, alone and in various combinations, and under different conditions, were studied by the same methods.

(3) The influence of both lecithin and cholesterin, alone and together under various conditions, on the protective power of serum from normal people and from cases of pernicious anemia was studied by quantitative measurements of protective power against hemolysis.

The method of making the quantitative measurements was the same as that outlined in an earlier paper on the study of the protective power of human serum against hemolysis by sodium oleate. A series of test-tubes, each containing 2 c. c. of a different dilution of hemolytic agent, was set up and to each tube of the series 0.25 c. c. of the substance to be tested for protective power was added. This was put in the water-bath at 37° C. for one-half hour, allowed to cool one-half hour, and then 0.25 c. c. of a 0.75 per cent suspension of guinea-pig cells was added to each tube. This preparation was incubated at 37° C. in the water-bath for two hours, allowed to sediment in the ice-box for an hour and then a reading of hemolysis in each tube was made. The dilutions of sodium oleate commonly used were from 1-45,000 to 1-90,000 in steps of 5000, thus: 1-45,000; 1-50,000; 1-55,000; 1-60,000; 1-65,000, and so on. With this method it was found, as reported in an earlier paper,[2] that serum from normal persons and patients with no anemia regularly protected guinea-pig corpuscles from hemolysis by sodium oleate up to strengths of 1-55,000 or higher, but that serum from patients with a hemolytic anemia or with an anemia and involvement of the liver or spleen rarely protected against strengths of sodium oleate beyond 1-75,000 and often only against solutions of hemolytic agent even weaker than this. Accordingly, in these experiments as in the earlier ones with serum the series of tubes carried dilutions of sodium oleate from 1-45,000 to 1-90,000 in steps of 5000. The technique of handling and the precautions to be taken in the preparation of the dilutions of sodium oleate, in the preparation of the guinea-pig cell suspension, in the cleaning of glassware, the method of titration, and the controls run, is presented in our earlier paper to which the reader is referred for details. The preparation of the materials tested for protected power will be given in this paper as experiments with them are taken up. When saponin was used as the hemolytic agent, it was found that normal serum protected guinea-pig corpuscles up to strengths of 1-18,000 to 1-20,000, and, as will be reported later in detail, the serum from those cases in which striking reduction in protective power against hemolysis by sodium oleate was found, protected against hemolysis by saponin only up to strengths of 1-26,000, 1-28,000 and 1-30,000. Therefore, with this weaker hemolytic agent saponin, as compared with sodium oleate, the series of tubes carried dilutions of 1-18,000 to 1-34,000 in steps of 2000. Other than the greater strengths of solutions used, the technique with the saponin was entirely the same as that previously detailed for sodium oleate.

In the technique outlined in our earlier paper, when serum was used as the protecting agent, it was always inactivated on the water-bath at 56° C. before being used. In some experiments presented in this paper, serum was used without previous inactivation and when so used this is stated. When no mention is made about this point the serum used had been inactivated.

I. Studies With Cholesterin Alone

All the cholesterin used in these experiments was extracted from gall-stones and was recrystallized three times. Three different methods of using it were employed:

(a) Cholesterin suspended in sterile normal salt solution was tested to see if it alone had any protective power against hemolysis, if it was inert, or if when so used it increased the hemolytic power of the hemolytic agent.

(b) Cholesterin was added to the hemolytic agents to determine what action it had on the hemolytic power exhibited by them.

(c) Cholesterin was added to serum to see if it increased or diminished the protective power of the serum.

(a) To determine the activity of cholesterin suspended in normal salt solution the cholesterin preparation was used in exactly the same way as serum in the titration for protective power against hemolysis as already described for serum. In the preparation of the cholesterin emulsion in salt solution, 10 milligrams of cholesterin were dissolved in 3 c. c. of a mixture of absolute alcohol 3 parts, ether 1 part. This was evaporated to 0.5 c. c. volume and then poured slowly and with constant stirring into 9 c. c. of sterile normal salt solution and the mixture brought up to 10 c. c. by the addition of more salt solution. A cloudy, milky fluid resulted. After standing overnight a white flocculent precipitate separated out, but when shaken the mixture took on the same milky appearance as when first made. For the test, 0.25 c. c. of this mixture was added to each tube of the hemolytic series just as serum diluted 1-20 was added to each tube in tests to measure quantitatively the protective power against hemolysis exhibited by a given serum. According to the figures of Bloor and Knudson[9] normal blood contains 175 to 200 milligrams of cholesterin per 100 c. c. If these values are correct, the 0.25 c. c. serum diluted 1-20, added to each tube of the hemolytic series, contained 0.025 milligrams or less of cholesterin. In the cholesterin preparation used in this titration the 0.25 c. c. of it added to each tube of the hemolytic series contained 0.25 milligrams of cholesterin. This is just ten times as much cholesterin as is contained in the serum added when tests are made to determine quantitatively its protective power. A parallel titration of the protective power of normal serum was carried out and at the same time the hemolytic series was set up with guinea-pig corpuscles unprotected by serum. In this last test the customary total volume in each tube of the series, 2.5 c. c., was made up by the addition of 0.25 c. c. of sterile normal salt solution instead of serum. By comparison with the titration of normal serum it was possible to determine whether the cholesterin preparation showed as much protection as the serum, and by comparison with the results of the test with unprotected guinea-pig cells, whether any protection was afforded by the cholesterin suspension, whether it was inert, or whether by its presence it increased the hemolytic power of the hemolytic agent.

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-160,000.

Cholesterin suspension in salt solution—Partial hemolysis up to and including dilution 1-170,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Cholesterin suspension in salt solution—Partial hemolysis up to and including dilution 1-40,000.

These experiments demonstrate that cholesterin suspended in normal salt solution affords no protection against hemolysis of guinea-pig corpuscles by sodium oleate. The slightly increased hemolysis seen in the titrations in which cholesterin in salt was used might have been due to the alcohol and ether in which the cholesterin was originally dissolved, a slight amount of which no doubt was introduced with the cholesterin suspension in salt solution. Cholesterin thus suspended in salt solution has marked protective power against hemolysis by saponin, but even in the relatively large amounts used does not protect as powerfully as normal serum.

(b) To determine what action cholesterin had when added to sodium oleate and saponin, cholesterin crystals were added directly to each and the usual titration of a normal serum for protective power was done with the hemolytic agents thus treated. Of a 1-1000 solution of sodium oleate and of saponin 100 c. c. were prepared as usual for the ordinary titration of protective power of serum and each was divided into two equal portions. To one of the 50 c. c. portions of sodium oleate and to a similar one of the saponin preparations 25 milligrams of cholesterin were added. The four preparations resulting, one of sodium oleate 1-1000, one of sodium oleate 1-1000 plus 25 milligrams of cholesterin, one of saponin 1-1000, and one of saponin 1-1000 plus 25 milligrams of cholesterin were kept in the incubator overnight and the further dilutions of hemolytic agent made the next morning as usual just before the test was to be done. In the morning the preparations to which cholesterin crystals had been added showed a white flocculent precipitate which diffused throughout the mixture with shaking. The only difference between these and the preparation of an ordinary titration for protective power against hemolysis was that there were not only hemolytic series of sodium oleate and saponin but also of sodium oleate and saponin to which cholesterin had been added the night before.

Titration of normal serum with each of these resulted as follows:

Sodium oleate:

Ordinary preparation—Partial hemolysis up to and including dilution 1-50,000.

Cholesterinized sodium oleate—Partial hemolysis up to and including dilution 1-45,000.

Saponin:

Ordinary preparation—Partial hemolysis up to and including dilution 1-20,000.

Cholesterinized saponin—Partial hemolysis up and including dilution 1-20,000.

These experiments show that cholesterin added as crystals to sodium oleate diminishes slightly its hemolytic power for guinea-pig corpuscles in the presence of normal human serum; and when added to saponin it does not influence its hemolytic power.

(c) To see whether cholesterin added to serum increased or diminished its protective power, 10 milligrams of cholesterin were added to 5 c. c. of serum and this was kept in the incubator overnight. The next day the serum showed no change in appearance and about the same amount of cholesterin crystals as had been added the day before was sedimented on the bottom of the tube. This tube was centrifuged and the serum free from crystals was pipetted off the top. Titrations of the protective power of this serum to which cholesterin crystals had been added, and then removed after a period of incubation, were carried out in the usual way and the results compared with those from the same serum to which no cholesterin had been added were as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Cholesterinized serum—Partial hemolysis up to and including dilution 1-55,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Cholesterinized serum—Partial hemolysis up to and including dilution 1-20,000.

From these results one may conclude that when cholesterin crystals are added directly to serum and later removed, the procedure does not influence its protective power against hemolysis by sodium oleate or saponin.

II. Studies With Lecithin Alone

The lecithin used in these experiments was a specimen supplied by the Digestive Ferments Company of Detroit and each time just before use was taken from the container in which it was received. The properties of lecithin in relation to hemolysis by sodium oleate and saponin were studied by the same methods used for cholesterin.

(a) Lecithin in salt solution was used in a quantitative titration for protective power against hemolysis.

(b) Lecithin was added to the hemolytic agents and the hemolytic power of these was tested.

(c) Lecithin was added to serum and the protective power against hemolysis of the serum thus treated was tested.

(a) When the action of lecithin in salt solution as compared with that of serum was studied, titrations for protective power against hemolysis were carried out in which lecithin in salt solution was added to the hemolytic series in exactly the same

manner as serum when similar studies already described were made with serum. To prepare the lecithin in salt solution 10 milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution and this 1-10,000 suspension was allowed to stand in the ice-box overnight. A slightly cloudy, yellowish fluid resulted. Of this suspension 0.25 c. c. was added to each tube instead of the serum diluted 1-20 as in tests for the protective power of serum, so that the dilution of lecithin in the final preparation in each tube of the hemolytic series was 1-100,000 (0.25 of a 1-10,000 lecithin suspension was put in each tube, the final volume of which was regularly 2.5 c. c. in the titration as set up). As with cholesterin, a parallel titration of the protective power of normal serum was carried out and at the same time a hemolytic series with guinea-pig corpuscles unprotected by serum was set up. The following results were obtained:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-180,000.

Lecithin preparation—Complete hemolysis up to and including dilution 1-180,000 almost immediately: that is, with less than half an hour's incubation. (In the usual test, hemolysis was recorded only after two hours' incubation and one hour in the ice-box.)

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Lecithin preparation—Partial hemolysis up to and including dilution 1-90,000.

The results of these experiments show that lecithin suspended in salt solution, when tested with a hemolytic series, does not protect against hemolysis by, but increases the hemolytic power of, sodium oleate. The same preparation does protect somewhat against hemolysis by saponin, but in the relatively large amounts used does not afford as much protection against saponin hemolysis as does normal serum.

(b) To determine what action lecithin exerts, when added directly to the hemolytic agents, the same methods were used as described above when similar tests were made with cholesterin. Twenty milligrams of lecithin were added to 50 c. c. of a 1-1000 sodium oleate solution and the same amount to 50 c. c. of a 1-1000 saponin solution. These preparations were allowed to stand overnight in the incubator and in the morning there was a slight yellowish-white precipitate which readily diffused through the mixtures with shaking, giving fluids of slightly cloudy, yellowish appearance. The results of the titrations of normal serum with hemolytic agents thus treated, compared with titrations of the same serum with the usual hemolytic agents, were as follows:

Sodium oleate:

Ordinary preparation—Partial hemolysis up to and including dilution 1-50,000.

Lecithinized sodium oleate—Partial hemolysis up to and including dilution 1-60,000.

Saponin:

Ordinary preparation—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized saponin—Partial hemolysis up to and including dilution 1-20,000.

These experiments show that lecithin added to sodium oleate even in the minute quantities used here increases somewhat its hemolytic power for guinea-pig corpuscles in the presence of normal human serum, but exerts no influence on the hemolytic power exhibited by saponin.

(c) To see whether lecithin added to serum influences its protective power against hemolysis, the same method of testing was again used as described above when similar tests were made with cholesterin; but instead of adding the lecithin directly to serum as was done with cholesterin, it was added in the salt solutions used to dilute the serum 1-20. Ten milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution and this lecithinized salt solution was used to make the 1-20 dilution of serum instead of sterile normal salt solution ordinarily employed. Of this preparation, 0.25 c. c. was added to each tube of the hemolytic series giving a concentration of lecithin of 1-105,000 in the final preparation. The titrations carried out with this lecithinized serum and with the same serum to which no lecithin had been added resulted as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-170,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-26,000.

These experiments show that lecithin added to serum decreases the protective power against sodium oleate markedly; or what is probably more nearly the correct interpretation, it increases the hemolytic power of the system by adding the hemolytic power of lecithin to that of the sodium oleate.*

* When one compares the results of the experiments where lecithin was added (1) to the sodium oleate and (2) to the serum, an apparent discrepancy is seen. The difference is explained by the fact that in the former, where hemolysis was increased slightly, the amount of lecithin present was much less than in the latter, where hemolysis was increased markedly. In the experiment in which lecithin was added to the sodium oleate, it was added to the 1-1000 stock solution and, therefore, when the greater dilutions were made from this, each tube of the hemolytic series contained decreasing amounts of lecithin as follows:

Sodium Oleate	Lecithin
1-50,000	1-125,000
1-55,000	1-137,500
1-60,000	1-150,000
1-65,000	1-162,500
Etc.	

In the experiment in which lecithin was added to the serum, however, the lecithin was in a concentration of 1-105,000 in each tube.

Also it appears that the protective power of the serum against saponin hemolysis is diminished.

III. Studies With Lecithin and Cholesterin Together

Cholesterin is not soluble in salt solution. It seemed possible that if cholesterin were suspended in the proper vehicle, it might protect against hemolysis by sodium oleate as it does against saponin when emulsified in salt solution. Lecithin was chosen for this purpose, since it is a solvent for cholesterin and moreover one which is widely distributed throughout the body and therefore presumably available for this purpose *in vivo*. In these experiments the same lecithin and cholesterin were used as in those detailed above. Two methods were employed:

(a) Lecithin suspended in salt solution and containing as much cholesterin as would go into solution was used in a quantitative titration for protective power against hemolysis.

(b) Serum to which lecithin had been added in minimal quantities and then as much cholesterin as would dissolve was tested for protective power against hemolysis.

(a) In making the preparation of both lecithin and cholesterin in normal salt solution to be tested for protective power against hemolysis, 10 milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution as in the test with lecithin alone, and a knife-point of cholesterin crystals was added. This was allowed to stand in the incubator overnight and the cholesterin crystals remaining were removed by filtration. The filtrate showed a moderately strong positive test for cholesterin, when tested by Liebermann's acetic anhydride-sulphuric acid method. This resulted in a 1-10,000 suspension of lecithin in normal salt solution containing as much cholesterin as would dissolve under these conditions. For this test 0.25 c. c. of this preparation was added to each tube in the hemolytic series as in all other similar titrations discussed above, giving a final concentration of lecithin 1-100,000 in each tube of the hemolytic series. Titrations of normal serum and a hemolytic series with guinea-pig corpuscles unprotected by serum were set up with the same hemolytic agents. The following readings were made:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Unprotected guinea-pig corpuscles—Partial hemolysis up to and including dilution 1-180,000.

Lecithin-cholesterin preparation in normal salt solution—Partial hemolysis up to and including dilution 1-180,000 (after one hour's incubation).

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig corpuscles—Partial hemolysis up to and including dilution 1-140,000.

Lecithin-cholesterin preparation in normal salt—Partial hemolysis up to and including dilution 1-60,000.

These experiments show that a 1-10,000 suspension of lecithin in normal salt solution containing cholesterin in solution does not afford any protective power against hemolysis by sodium oleate, but that hemolysis is increased. This increase is probably due to the lecithin added, for as shown above, lecithin introduced adds to the hemolytic power of the system. With lecithin-cholesterin preparation in normal salt solution, however, the increase in hemolysis is not quite so much as when lecithin alone is used. The correct interpretation of this probably is that the cholesterin inhibits a part of the added hemolysis due to the lecithin present. From these results it also appears that the lecithin-cholesterin salt preparation exerts a rather marked protective power against hemolysis by saponin, but even in the relatively large amounts employed does not protect nearly as strongly as normal serum.

(b) To test how the addition of both lecithin and cholesterin to normal serum would influence its protective power against hemolysis, one point had to be established first. It has already been shown that when lecithin was added to serum in such quantities that in the final preparation there was a concentration of 1-100,000 of lecithin in each tube of the hemolytic series, the protective power was diminished. The point to be determined was whether or not lecithin could be added to the serum in such small quantities that, when a titration was done, it did not react towards sodium oleate differently than normal serum. A series of tests showed that, if lecithin was added to the serum in such quantities that in the final preparation in each tube of the hemolytic series it was in a concentration of 1-300,000 or less, the results with sodium oleate did not differ from those obtained when normal serum was used.* Cholesterin crystals were added to this 1-30,000 lecithin solution and were allowed to remain in contact with the solution overnight in the incubator. The cholesterin crystals remaining were then removed by centrifugation. The supernatant fluid contained cholesterin in solution as shown by a positive Liebermann's test. This material was used to dilute the serum 1-20 instead of normal salt solution just as in the experiments with lecithin alone, and titrations for protective power against hemolysis of serum thus treated were made. At the same time titrations of normal serum and of serum to which lecithin had been added in the small quantity shown not to influence its reaction with sodium oleate were done. The results obtained were as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-55,000.

* The amount of lecithin which could be added to serum without lowering its protective power for saponin was not determined. The amount of lecithin, which, added to serum, did not increase the hemolysis seen with sodium oleate, did, however, increase the hemolysis with saponin. This is surprising, because lecithin in the absence of serum, protects slightly against saponin hemolysis, whereas with sodium oleate the amount of hemolysis is increased. This finding was the same in several duplicate experiments at different times. No explanation of this apparent paradox has so far presented itself.

Serum to which lecithin and cholesterin had been added—Partial hemolysis up to and including dilution 1-50,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-26,000.

Serum to which both lecithin and cholesterin had been added—Partial hemolysis up to and including dilution 1-20,000.

The results obtained in these experiments show that when lecithin, in such small amounts that it does not increase hemolysis with sodium oleate, is treated with cholesterin and this mixture is added to serum, the protective power of the serum against hemolysis by sodium oleate is increased, but only to a slight degree. The same amount of lecithin, which added to serum does not increase the hemolysis seen with sodium oleate but does increase hemolysis with saponin, when combined with cholesterin, affords some protection against saponin as compared with a solution of lecithin alone of similar strength in normal salt solution. In this case again, the correct interpretation probably is that the cholesterin merely neutralizes the increased hemolysis due to the lecithin present.

IV. Studies With Cases of Pernicious Anemia

In experiments already discussed, lecithin alone, cholesterin alone, and lecithin and cholesterin together were added to normal serum which had been inactivated and the effect on the protective power of the serum against hemolysis by sodium oleate and saponin recorded. Exactly the same technique being employed, similar experiments were carried out with serum from patients with pernicious anemia. The results were the same as those obtained when normal serum was used. Cholesterin added to serum afforded no increased protective power against sodium oleate or saponin. Lecithin in a strength of 1-30,000, and containing as much cholesterin as it would dissolve added to serum gave no increased protection against sodium oleate or saponin.

V. Studies With Serum That Had Not Been Inactivated

All the experiments detailed above were carried out with serum that had been inactivated. It appeared possible that the activity of normal ferments of the blood might be necessary before cholesterin added to serum could act as an antihemolytic agent. Accordingly, cholesterin was added to sera that had not been inactivated, from normal persons and from patients with pernicious anemia of different degrees of severity. Titrations carried out with these sera showed, however, that they did not react differently than inactivated serum to which cholesterin had been added.

VI. Studies With Serum After Extraction With Petroleum Ether

In further attempts to correlate two of the findings in pernicious anemia, the diminished cholesterin in the blood and the lowered protective power against hemolysis by sodium oleate, quantitative estimations of the protective power of serum against hemolysis by sodium oleate and saponin were carried out after an attempt had been made to remove the cholesterin from serum by extraction with petroleum ether. The petroleum ether extract was similarly tested. The petroleum ether used in the extractions was re-distilled at a temperature below 56° C. before use. Five cubic centimeters of serum were extracted vigorously in a continuous extractor for 12 hours at a temperature kept constantly below 56° C. When the petroleum ether used in the extraction was tested for cholesterin by Liebermann's reaction, it gave a strongly positive test. All the cholesterin had not been removed from the serum, however, for when the extracted serum was similarly tested, a trace of cholesterin was found to be present.

(a) The serum thus extracted was heated for two hours on the water-bath at 56° C. to remove all traces of petroleum ether. It was then used in a titration of protective power against hemolysis just as was done with normal serum. The results obtained from this and a titration of normal serum done at the same time were as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-60,000.

Extracted serum—Partial hemolysis up to and including dilution 1-105,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Extracted serum—Partial hemolysis up to and including dilution 1-34,000.

The results of these experiments show that, when serum is extracted with petroleum ether, there is a marked diminution in the protective power against hemolysis by sodium oleate and saponin.

It seemed possible that the increased hemolysis observed, when extracted serum was used to protect against sodium oleate and saponin, might not be due to a reduction in the protective power of the extracted serum but rather to an increase in the hemolytic power of the series because of a trace of petroleum ether, an active hemolytic agent, possibly remaining in the serum when used. The control tube set up with each titration to show that the serum used was not hemolytic in itself was negative in this instance as in all others, indicating that any trace of petroleum ether possibly in the serum was not in sufficient concentration to influence the hemolysis in the test. To control this point further, however, the following experiment was carried out:

Three drops of the same lot of petroleum ether used in the extraction were added to 1 c. c. of serum and a titration of this serum for protective power was made.

This titration resulted as follows:

Normal serum showed with sodium oleate—Partial hemolysis up to and including dilution 1-55,000.

Normal serum plus petroleum ether showed with sodium oleate—Partial hemolysis up to and including dilution 1-60,000.

Furthermore, this serum to which petroleum ether had been added showed a negative control tube, indicating that the serum itself as used had no hemolytic properties.

In view of these findings one is justified in concluding that the greatly increased hemolysis resulting when extracted serum was used was not due to any trace of petroleum ether which might possibly have been present in the serum when titrated.

(b) To study the petroleum ether extract for protective power against hemolysis, the petroleum ether used in the extraction of the serum and shown to contain cholesterin, was evaporated to dryness. The residue was taken up by boiling with successive portions of a mixture of absolute alcohol three parts and ether one part, and the combined portions were evaporated to 1 c. c. Half a cubic centimeter of this was added to 9 c. c. of sterile normal salt solution slowly and with constant stirring, and 0.5 c. c. was added to 9 c. c. of normal salt solution containing lecithin in a strength of 1-30,000. Each portion probably contained then much, if not most, of the cholesterin from 2.5 c. c. of serum, and one of them in addition the amount of lecithin which had been shown to be without influence in itself on the reaction of serum with sodium oleate. For this test 0.25 c. c. of each preparation was added to each tube of the hemolytic series in exactly the same way as serum in the usual test for the protective power of serum. At the same time the customary titration of normal serum and a hemolytic series with guinea-pig corpuscles unprotected by serum were set up for comparison. The following results were obtained:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-60,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-180,000.

Petroleum ether extract—Partial hemolysis up to and including dilution 1-180,000.

Petroleum ether extract to which lecithin had been added—Partial hemolysis up to and including dilution 1-180,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Petroleum ether extract—Partial hemolysis up to and including dilution 1-40,000.

Petroleum ether extract to which lecithin had been added—Partial hemolysis up to and including dilution 1-24,000.

The results of these titrations demonstrate that the residue from evaporation of the petroleum ether used in extracting serum, when taken up in alcohol-ether and suspended in salt solution has no protective power against hemolysis by sodium oleate either alone or after lecithin had been added. The petroleum ether extract, when similarly treated, has considerable protective power against hemolysis by saponin and this is increased in the presence of lecithin.

In summary, several points of interest have been demonstrated in these experiments.

(1) Cholesterin emulsified in salt solution does not protect against hemolysis by sodium oleate but does protect against hemolysis by saponin. However, when cholesterin, without previous solution in alcohol-ether and suspension in salt solution, is added as crystals to serum, either inactivated or not inactivated, and the crystals removed after a definite period, it does not increase the protective power of the serum against hemolysis by sodium oleate or saponin; and if added as crystals to either of these hemolytic agents, it does not diminish their hemolytic power.

(2) Lecithin suspended in salt solution increases the hemolysis seen with sodium oleate but protects slightly against hemolysis by saponin. In the presence of serum, however, lecithin increases the hemolysis seen with both sodium oleate and saponin.

(3) A relatively strong suspension of lecithin in salt solution and containing cholesterin in solution does not protect against hemolysis by sodium oleate but does afford protection against saponin. When a weak suspension of lecithin in which cholesterin has been dissolved is added to serum, the protective power of the serum against sodium oleate and saponin is not increased.

(4) Cholesterin and lecithin, alone and together, added to serum from cases of pernicious anemia do not react differently in relation to hemolysis by sodium oleate and saponin than when added to normal serum.

(5) Serum, when vigorously extracted by petroleum ether at a temperature constantly below 56° C., loses most of its protective power against hemolysis by sodium oleate and saponin. The residue, after evaporation of the petroleum ether used in the extraction of serum, reacts just as cholesterin in relation to hemolysis by sodium oleate and saponin; *i. e.*, when taken up in an alcohol-ether mixture and emulsified in normal salt solution, or in normal salt solution containing lecithin, it affords no protection against hemolysis by sodium oleate, but does protect against saponin hemolysis.

The results obtained in these experiments indicate that there is much to be learned concerning the part played by cholesterin and lecithin in hemolytic processes and in particular, the hemolytic processes which may occur in the body. They do not permit one to suggest any possible relationship which cholesterin and lecithin may have to the hemolysis occurring in the hemolytic anemias.

Conclusions

(1) The protective power of the serum normally present against hemolysis by sodium oleate and shown to be diminished in hemolytic anemia has not been shown to be directly dependent upon:

(a) Cholesterin alone.

(b) Lecithin alone.

(c) Simple combinations of lecithin and cholesterin as used in these experiments.

(2) The protective power of serum normally present against hemolysis by saponin, also diminished in hemolytic anemias, has not been shown to be wholly dependent upon:

(a) Cholesterin alone.

(b) Lecithin alone.

(c) Simple combinations of lecithin and cholesterin as used in these experiments.

(3) The diminished protective power against hemolysis of the serum in hemolytic anemia has not been explained simply on the basis of the diminution in the total cholesterin of the blood found in these conditions.

BIBLIOGRAPHY

1. Ford, W. W.: The Toxins and Antitoxins of Poisonous Mushrooms (*Amanita phalloides*). J. Infect. Dis., Chicago, 1906, III, 191.

1. Moss, W. L.: Studies on Isoagglutinins and Isohemolysins. Johns Hopkins Hosp. Bull., Balt., 1910, XXI, 63.

1. Sellards, A. W.: The Hemolytic Action of Bile and Its Inhibition by Blood Serum. Johns Hopkins Hosp. Bull., Balt., 1908, XIX, 268.

1. Moss, W. L., & Barnes, F. M.: Concerning the Much-Holzmann Reaction. Johns Hopkins Hosp. Bull., Balt., 1911, XXII, 278.

1. Noguchi, H.: Ueber gewisse chemische Komplementsubstanzen. Biochem. Ztschr., Berl., 1907, VI, 327.

1. v. Liebermann, L.: Ueber Hämagglutination und Hämatolyse. Biochem. Ztschr., Berl., 1907, IV, 25.

1. Lamar, R. V.: Chemo-immunological Studies on Localized Infections, 1st paper. J. Exper. Med., Balt., 1911, XIII, 1.

1. Lamar, R. V.: Chemo-immunological Studies on Localized Infections, 3d paper. J. Exper. Med., Balt., 1911, XIV, 256.

2. Clark, H. M., & Evans, F. A.: One Factor in the Mechanism of Hemolysis in Hemolytic Anemia. Johns Hopkins Hosp. Bull., Balt., 1920, XXXI, 354.

3. Noguchi, H.: On Certain Thermostabile Venom Activators. J. Exper. Med., Balt., 1906, VIII, 87.

4. Bauer, F.: Eine besondere Reaction in Nabelschnurblute Neugeborener. Mitteilung zur Muchschen Psychoreaction. München. med. Wchnschr., 1909, 56-2, 1367.

5. Arrhenius, S.: Hämolytische Versuche. Biochem. Ztschr., Berl., 1908, XI, 161.

6. Bayer, G.: Untersuchungen über die Gallenhämolyse. Ueber die Hemmungswirkung normaler Sera. Biochem. Ztschr., Berl., 1907, V, 368.

7. Ransom, F.: Saponin und sein Gegengift. Deutsche med. Wchnschr., Berl. & Leipz., 1901, XXVII, 194.

8. v. Liebermann, L. und v. Fenyvessy, B.: Ueber die gegenseitige Activierung hämolytisch unwirksamer Stoffe. Ztschr. f. Immunitätsforsch. u. exper. Therap., Jena, 1909, II, 436.

9. Bloor, W. R., & Knudson, A.: Cholesterol and Cholesterol Esters in Human Blood. J. Biol. Chem., Balt., 1917, XXIX, 7.

10. Gorham, F. D., & Myers, V. C.: Remarks on the Cholesterol Content of Human Blood. Arch. Int. Med., Chicago, 1917, XX, 599.

11. Denis, W.: The Influence of Splenectomy on Metabolism in Anemia. Arch. Int. Med., Chicago, 1917, XX, 79.

VARIATIONS IN THE BACTERIAL FLORA OF THE UPPER AIR PASSAGES DURING THE COURSE OF COMMON COLDS

By ARTHUR BLOOMFIELD

(From the Biological Division of the Medical Clinic, The Johns Hopkins University and Hospital)

INTRODUCTION

Despite the frequency and prevalence of the common cold remarkably little accurate information is available bearing on the exact nature and cause of this malady. Yet this is not surprising, for the very mildness of the disease, as a rule, keeps the patient from seeking medical aid until some grave complication supervenes. Furthermore, even if the physician is consulted, the circumstances are usually not conducive to careful study and observation, since the patient is ambulant, and is not seen under conditions which promote the keeping of careful records. Finally, the mildness of the symptoms makes exact definition of the disease difficult, and the primary cold is often confused with sinus infections, tonsillitis, laryngitis, or other complicating disturbances of the upper air passages.

None the less it seems of the utmost importance that the problem of the common cold be solved. Although one does not die of this disease, the total morbidity is tremendous—few people go through a winter without at least one acute coryza, and the sum total of inconvenience to a community from this cause can hardly be overestimated. In addition, a cold is often the starting point for a variety of more serious and intractable disturbances, such as sinus and middle-ear infections, laryngitis and bronchitis. Furthermore, it seems quite possible that an acute coryza may be related to the development of carrier states involving organisms which may cause even more serious diseases, such as pneumonia and meningitis; in fact the ultimate pathological sequences of the cold may be vastly more extensive than one would at first sight suppose. Finally, were the cause and nature of this malady elucidated much important knowledge on epidemiological matters might be obtained, and the study of other respiratory diseases, such as influenza, might be simplified.

During the past winter we have made certain bacteriological studies on colds which we desire to present, but it seems advisable first to review the literature and to discuss the clinical features of the disease.

CLINICAL NOTES

In the study of any disease various lines of attack may be pursued. If the causal agent be definitely ascertained, special bacteriological or other methods may suffice in selecting cases which comprise one entity, but in the absence of such knowledge more advance may perhaps be made by careful clinical observation of the natural history of the disease, and by attempting to relate it to other conditions generically similar

but specifically different. Thus, in the recent epidemic of influenza, pure bacteriological studies led merely to confusion, whereas clinical observation differentiated the primary disease from its picturesque but unessential complications, and drew a definite analogy between it and certain other diseases of unknown etiology such as measles. It seems worth while, therefore, to present a brief analysis of the disease—acute coryza—from the clinical standpoint.

In uncomplicated cases we recognize two sets of phenomena—the constitutional reaction, and the local disturbances in the upper air passages. Even in mild cases the former are rarely lacking—malaise, chilly sensations, headache or general aching, and slight fever. The local reaction manifests itself by dryness, rawness or burning of the mucous membranes of the nose, pharynx or palate, which is soon followed by some swelling of the parts, as evidenced by nasal occlusion, sneezing and lachrymation, and by more or less watery discharge. In simple cases the constitutional reaction subsides in a few days, and the nasal and pharyngeal discharge, after becoming more abundant and mucoid, disappears. The patient is well, but the mucous membranes may feel raw or dry for days or even a few weeks. In uncomplicated cases there is never any local pharyngeal or tonsillar exudate or any really purulent discharge; the local condition seems to consist simply of a hyperemia and swelling of the mucous membranes. About 20 cases were available for study in the records of The Johns Hopkins Hospital and from these histories the following information was obtained—fever, if present, never lasts more than a few (two or three) days, the leucocytes are diminished, or at least not increased, and the duration of uncomplicated cases is definitely limited.

In summary, then, the uncomplicated cold is a mild disease manifesting itself by a constitutional reaction and by hyperemic phenomena of the upper air passages without local suppurative complications. The duration is brief and there is a tendency to leucopenia.

This primary disease is to be sharply differentiated from certain local complications which occur frequently, and often overshadow the picture, although they are by no means essential but are distinctly secondary. The clinical sequence suggests that the cold itself prepares the ground in some way so that local complications due to pyogenic organisms may arise, and indeed, such complications rarely manifest themselves until the initial disease has been present at least several days. Sinus infections, middle-ear infections, laryngitis, tracheitis and bronchitis, occur frequently—pneumonia and sepsis rarely follow a simple cold. Such processes, once established, may flare up promptly during a subsequent cold and thus serve still further to confuse the picture.

We have then in the case of the cold a perfect analogy to what obtains in epidemic influenza—a primary disease relatively mild in itself, but creating an almost specific predisposition to certain complications in the respiratory tract. The importance of this differentiation between primary disease and complication cannot be overestimated, inasmuch as the bacteriological findings associated with the former and with the latter must be distinguished.

In regard to epidemiology two outstanding facts may be mentioned—the disease is essentially a seasonal one, and one which affects groups of people. At the same time the details of transmission are entirely obscure. Colds are clearly contagious, as evidenced by rapid spread through groups of people, and yet susceptible individuals intimately exposed may escape infection. Similar contradictions have been observed in influenza.

In regard to immunity there is little to be said. Occasional individuals seem to be immune to colds, and they never develop the infection. Others seem highly susceptible and have many attacks during the course of one season. Whether a transient protection is conferred by an attack, we do not know—certainly such protection is usually very temporary.

In summary, then, the common cold falls into a rather sharply defined group of diseases including (1) the common cold, which is endemic and at times mildly epidemic; (2) true influenza which is pandemic; and (3) so-called grippe, intermediate in severity between the cold and influenza, and usually occurring in mildly epidemic form. All these diseases have the following common characteristics:

1. There is a primary disease manifested by a constitutional reaction and by hyperæmic phenomena in the upper air passages.

2. The uncomplicated disease is of self-limited duration.

3. There is usually leucopenia or at least an absence of leucocytosis.

4. Spread of the disease seems to be mainly by direct contact.

5. There is a remarkable tendency to complications in the respiratory tract; in the case of colds to localized infections, such as otitis, sinusitis, laryngitis and bronchitis; in influenza, to broncho-pneumonia as well.

Our purpose in presenting this clinical analysis is to bring out clearly the fact that the common cold is a definite disease of fixed characteristics, to be distinguished sharply from complications which frequently occur during its course. This fact, as well as its striking resemblance to a group of diseases of unknown etiology, should make one cautious in accepting any of the obvious organisms of the throat as its cause.

Literature

The literature on the subject of colds is scant. Even the textbooks have not thought the disease worthy of serious consideration. We find no mention of it, for example, in Reynolds' *System of Medicine*, the standard English work of 50 years ago, or in the more modern system of Osler and McCrae. Mohr and Staehelin are silent on the question, and in Albutt's system a brief and incomplete note is found under the heading of acute rhinitis. In Osler's *Practice* alone have we found anything approaching an adequate discussion of the disease, which the author describes with his usual clearness and accuracy under the heading of acute catarrhal fever.[1] The explanation of this general neglect of so common a malady seems to us to be due again to the lack of general recognition of the common cold

as a definite entity. Almost always the primary disease and its complications are confused. The terminology for example, falls under two heads—first a series of designations which indicate the disease as a whole, and secondly, terms which apply to one phase or complication only. In the former group we find "the cold," "the common cold," "acute catarrh," "acute coryza," and "acute catarrhal fever"; and in the latter "acute rhinitis," "acute pharyngitis," "acute laryngitis," etc. Fantus [3] recently has suggested that colds, grippe and influenza, all be included under the general heading of the catarrhal fevers. There seems to us much reason for such a designation, as it points out the undoubted generic relationship between these various conditions. It would seem wise in the present state of our knowledge to mention both the primary disease and the complication (if present) in writing the diagnosis; for example, "acute catarrhal fever—sinusitis," or "acute catarrhal fever—broncho-pneumonia," whereas in simple cases one would simply say "acute catarrhal fever."

Views as to the Nature of Colds

Aside from bizarre and untenable theories as to the nature of the common cold, usually put forward by a single individual and never gaining general recognition, three main views have prevailed as to the nature of this affection. The first, which was almost universally accepted until the bacteriological era became well established, presumed that the cold was indeed due to exposure to cold or change of temperature in some form. So firmly was this idea fixed in the lay and professional mind that a period of a one hundred years could pass without producing any modification of this doctrine. In 1786, for example, Thomas Hayes in a little treatise entitled "A Serious Address on the Dangerous Consequences of Neglecting Common Coughs and Colds" [4] says "A cold arises from the effect of cold or moist air, applied to the surface of the body and lungs, from going too thinly clad, or exposing the body to cold air, after having been heated by exercise; or when the pores are opened from drinking warm liquors." There follows an admirable clinical description in which the primary disease is well differentiated from its sequels. "A cold, then, is a sense of chilliness on the skin attended with a lassitude and weariness, and slight shivers at times, with a degree of headache, and flying pains in the small of the back and limbs, a stuffing of the nose with or without dry tickling cough or hoarseness. Sometimes the sneezing, stuffing of the nose, or cough, give the first intelligence of its approach, and sometimes it is preceded by some of the other symptoms." One hundred years later Carpentier [5] gives as causes of the common cold—use of tobacco, occupation attended with dust, smoke or irritating gases, excessive dryness or moisture of the atmosphere, sudden changes of temperature, or getting wet feet. "A very pernicious habit is wetting the head, which is practiced daily by young ladies." Exposure to night air, damp clothes, going without a collar, cravat, or cuffs, facing the wind in traveling and not having nose, mouth, throat and chest protected with extra wraps are also listed. Furthermore, certain constitutional causes such as rheumatism and neurasthenia are mentioned. At about the same time Lewisohn [6] claims that colds are due to indigestion, and Ewing [8] that their cause is "interference with nutrition in some part of the body." Hitz [7] finds atmospheric changes a direct factor in the etiology of catarrhal disease of the respiratory tract, and Jack,[8] more recently and to our mind unconvincingly, tries to relate colds to temperature, moisture, and soil conditions.

Although modern writers no longer regard cold as the sole and complete cause of catarrhal affections of the upper air passages, the relationship of exposure and chilling to disease is still the subject of consideration. Schade [9, 10, 11] reports extensive and interesting clinical and experimental observations on the effect of cold on tissues, and its relation to disease. Certain definite effects on the mucous membranes of the respiratory passages seem to be proven. These are reflex vasomotor changes which may lead to anemia or hyperæmia, and also direct irritative phenomena. Schade describes interesting physical changes in tissues which have been affected by cold. In the case of the respiratory mucosa exposure to cold may, according to this observer, produce a so-called "cold catarrh." Its features are onset during or directly after the cold trauma, with rawness and secretion. Objectively, the mucous membrane may appear reddened. There is no fever and the disturbance, which is to be regarded as purely local, lasts only one or two days. Schade believes also that this cold catarrh may allow bacterial invasion with secondary effects. His ideas are supported by an epidemiological study on 17,000 men who were in the trenches on the western front from November 14 to May 17. The first two winters were mild, but during the third, which was severe, the incidence of respiratory infection increased four-fold.

Miller and Noble [12] review the experimental work on the relation of cold to infection and report experiments of their own to the effect that respiratory infection of rabbits with bacillus bovisepticus (snuffles) is favored by chilling, after the animals have been accustomed to heat. Mudd and Grant,[13] and Grant, Mudd and Goldman,[14] showed that chilling of the body surfaces causes vaso-constriction and ischemia in the mucous membranes of the palate, pharynx, and tonsils. In a few cases the flora of the pharynx and tonsils was studied before and after chilling, and apparently there was some increase in certain organisms following the procedure.

On the whole, then, it seems clear that cold may produce an irritation of the mucous membranes of the upper air passages. It also seems quite possible that this injury may be one of a group which alters conditions in such a way as to allow secondary bacterial invasion. How real a part it plays in the production of the true "common cold" cannot be absolutely decided at present, but surely it can only be a contributary factor of the most general sort when one considers the facts of epidemiology and the clinical features of the disease.

With the development of bacteriological knowledge opinion gradually inclined toward infection as playing a part in the production of colds. The effect of cold was still considered important, however, as a predisposing factor following which a bacterial invasion occurred by organisms present in the mouth. James [15] for example, states that colds are caused by

exposure to cold or irritation of the nasal mucous membranes by dust or gas which allows the entry of mouth bacteria. Haig[16] claims that "a cold is excess of uric acid in the blood, plus local action of cold, plus a microbe. The microbe by itself can do nothing"; and Walsh[17] believes that a cold is due to bacterial invasion by organisms present in the mouth following cold or some other injury. This general view seems to have been very popular until recently, and was quite generally accepted. While it marked a distinct advance over the old idea that cold alone was the causal factor, it can hardly, as we shall point out later, explain the facts of epidemic colds.

During the recent years of bacteriological enthusiasm cold has been almost entirely lost sight of in considering the etiology of acute catarrh, and a primary and complete rôle has been ascribed to various bacteria. All of these studies may be criticized for several reasons. In the first place no clinical distinction is made between the primary cold and its complications with consequent confusion of bacteriological results, secondly, no adequate control studies of the flora under normal conditions have been made, and thirdly, the attention of the investigator has very often been confined to only one organism. Cecil[18] for example, in working out the incidence of S. viridans in acute respiratory infections found this organism predominatingly in a large percentage of colds. He fails to recognize it as a normal mouth inhabitant and seems unaware that plates from healthy individuals may often show this organism in almost pure culture. Similarly, Voorhees[19] states that "colds are due to pneumococcus, catarrhalis and other organisms," Mackay[20] finds pneumococcus, B. influenzæ, catarrhalis, staphylococcus, streptococcus and diphtheroids—in other words the entire normal flora of the mouth. Tunnicliff[21] describes a small anaërobic organism, and Floyd,[22] in a study of the streptococcus and pneumococcus groups as found in relation to acute upper respiratory tract infections, encountered these organisms frequently in colds. He makes no attempt to differentiate primary disease and secondary complications, nor does he seem familiar with the normal variations in flora. As far back as 1902 Pfeiffer[23] ascribed colds to the micrococcus catarrhalis which he isolated from catarrhal infections.

In addition to the usual bacteria, filtrable viruses have been discussed as a possible cause of colds. Kruse[24] first brought out this idea. Secretions blown from the nose of a patient with an acute cold were diluted 15 times with salt solution, filtered through a Berkefeld filter and the filtrate was instilled into the nasal passages of 36 healthy people. After an incubation period of two or three days 15, or 42 per cent, of these individuals developed typical colds. Foster,[25, 26] following Kruse and using a similar technique, produced typical colds in at least seven of ten healthy men. After an incubation period of from six or eight to 30 hours dryness of the nose and throat, sneezing, headache, chilliness, flushing, and rhinorrhœa (second day), developed. In six cases there was slight fever—up to 100° F. Other symptoms were sore throat (five cases), pain in the back (four cases), herpes (one case). The duration of the experimental disease was from three to six days. By anaërobic (ascites-tissue) methods Foster cultivated a minute organism, which in turn produced colds on inoculation. Foster's protocols are quite convincing, and one wonders that more effort has not been made by other workers to confirm and extend his work.

Summary

In summary, then, a consideration of the clinical features of colds together with the information available in the literature leads to the following conclusions:

1. The common cold is a definite disease generically related to grippe and influenza. The primary disease is often followed by local complications which tend to overshadow the picture.

2. Cold may produce disturbances in the upper air passages which are to be distinguished from true infectious colds.

3. None of the common bacteria found in the nose or throat have been proven to be the primary cause of colds.

4. The most convincing evidence in the literature favors a filtrable virus as the cause of the common cold.

Present Observations

In a previous paper[27] we reported studies on the normal (aërobic) flora of the throat in healthy individuals. This flora was found to be relatively simple, and practically constant over considerable periods of time. With the background thus obtained it seemed desirable to study respiratory infections to discover what deviation from the norm might be present under conditions of disease. The present report deals with the findings in a series of cases of acute coryza (common cold).

Material

Ten individuals were studied during the months of November and December 1920. At this time colds were very prevalent in Baltimore and the rapid spread of the disease, together with the clinical features, made it clear that we were dealing with the so-called infectious type of acute coryza. Most of our patients had the disease in its uncomplicated form, a few developed secondary local complications. The clinical features will be discussed in detail in connection with the protocols.

Methods

The same methods as those used in studying the normal flora were employed in the present work. Cultures were made at intervals during the active stage of the disease and during convalescence on rabbit-blood agar and oleate hæmoglobin agar. The swabs were taken from the naso-pharynx and tonsils. Cultures were not made from the nose, because we desired to compare the conditions with those previously studied in normals. Furthermore, in addition to draining the nasal mucous membranes, the naso-pharynx was involved in all cases.

The cultures were worked out in considerable detail both quantitatively and qualitatively.

PROTOCOLS

CASE I.—SL

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic Streptococcus	Pneumococcus	Remarks	Clinical notes
Jan. 4...	∞ At least two varieties.	∞	100 cols. (hemolytic).	0	0	Many cols. of a minute Gram-positive organism. A few cols. Staph. albus.	Cold began Jan. 2, nose occluded, throat raw. Tonsils removed. Nasal and pharyngeal mucous membranes reddened.
Jan. 7...	∞	∞	Many cols. (hemolytic).	0	0	.....	Convalescent.
Jan. 10..	∞	∞	0	0	0	A few cols. Staph. albus.	Well.

Comment.—Clinically, the case was one of an uncomplicated cold. The members of the normal flora—non-hemolytic streptococcus and Gram-negative cocci were present in large numbers in all the cultures. Staph. albus was present in small numbers in the first and third cultures. In the first culture there were many colonies of a minute Gram-positive organism. Hemolytic influenza bacilli were present in the first two cultures. Our impression of the bacteriology of this case is that we are dealing with the normal flora plus transient insignificant organisms such as one finds in healthy controls. It does not seem possible to assign a causal rôle to any of the bacteria found. The significance of the hemolytic influenza bacillus will be discussed later.

* ∞ = innumerable.

CASE II.—C

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 12..	∞	∞	100 cols. (hemolytic).	0	0	A few cols. Staph. albus.	Cold began Nov. 12. Stuffiness in nose, sneezing, slight headache, and slight sore throat. No discharge. Exam.: Nose—mucous membrane slightly red and swollen. Throat slightly red.
Nov. 15..	∞	∞	0	0	0	.....	Condition about the same.
Nov. 17..	∞	∞	20 cols. (hemolytic).	0	0	.....	Feels well.

Comment.—Clinically, the case is one of an uncompleted cold. The bacteriological findings are analogous to those in Case I.

CASE III.—FR.

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 3...	A few cols.	∞	Many cols. (hemolytic).	0	Many cols.	Many small dewdrop colonies of a Gram-positive coccus. Culture of washed nasal secretion: 6 cols. Staph. albus.	Fourth day of cold. Stuffiness in nose, rawness of throat, marked malaise. Today nose is occluded, the mucous membrane is reddened, pharynx is red. Pale green muco-pus from nose.
Dec. 10..	Many cols.	∞	A few cols. (hemolytic).	0	Many cols.	A few cols. of a Gram-positive diphtheriod.	Practically well. Mucous membranes still a little raw.
Dec. 13..	Many cols.	∞	50 cols. (hemolytic).	0	Many cols.	Many cols. of a hemolytic staphylococcus.	Well.
Dec. 29..	∞	∞	25 cols. (hemolytic).	0	0	.....	Well.

Comment.—Clinically, the case is one of an uncomplicated cold. The normal flora—non-hemolytic streptococcus, and Gram-negative cocci—were present throughout, and also a hemolytic influenza bacillus. Pneumococci were present in the first three cultures. A variety of bacteria were temporarily present. The only organism which might possibly be regarded as causal was the pneumococcus present in the first three cultures, but inasmuch as it is frequently found in the mouth and was rarely obtained in the other cases, one can hardly incriminate it as the cause of colds in general. Furthermore, culture from the nasal discharge showed only Staph. albus and no pneumococci.

PROTOCOLS—CONTINUED

CASE IV.—ERB

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 20..	∞	∞	Many cols. (hemolytic).	0	0	A few cols. Staph. albus.	Onset November 19. Feels badly, throat raw. On exam. pharynx is distinctly reddened.
Nov. 22..	∞	∞	A few cols. (hemolytic).	0	0	.....	Full-blown cold. Feels badly, eyes and nose congested. Throat raw, and looks red.
Nov. 23..	Many cols.	∞	Many cols. (hemolytic).	0	0	3 cols. of a yellow hemolytic staphylococcus. ∞ small dew-drop colonies of a Gram-pos. diplococcus.	Condition unchanged.
Nov. 29..	∞	∞	A few cols. (hemolytic).	0	0	Many small dew-drop colonies of a Gram-pos. diplococcus. 20 colonies of a "spreader," many colonies of a yeast.	Condition much better. Throat still feels raw. Appearance of throat about normal.
Dec. 2...	Many cols.	A few cols.	200 cols. (hemolytic).	0	0	100 cols. of a Gram-pos. diphtheroid. ∞ small dew-drop colonies of a Gram-pos. diplococcus. ∞ Cols. of yeast.	Exacerbation yesterday, feels badly, nose completely occluded, eyes running, moderate amount of mucoid discharge from nose.
Dec. 4...	∞	A few cols.	20 cols. (hemolytic).	0	0	A few small dew-drop colonies of a Gram-pos. diplococcus Many cols. of a Gram-pos. diphtheroid. ∞ cols. of a yeast.	Condition unchanged.
Dec. 10..	∞	∞	50 cols. (hemolytic).	0	0	A few small dew-drop colonies of a Gram-pos. diplococcus A few small tough colonies. of a minute Gram-positive organism.	Practically well. Mucous membranes still raw.
Jan. 3...	Many cols.	∞	100 cols. (hemolytic).	0	0	A few small tough colonies of a minute Gram-pos. organism.	Well.

Comment.—Clinically, the case is one of a severe but uncomplicated cold. The bacteriological findings are of special interest. The normal flora as well as a hemolytic influenza bacillus were present in all the cultures. Staph. albus, Staph. aureus, and a spreader were present as transients in a single culture. A Gram-positive diphtheroid was present on two successive cultures but not before or afterwards. A minute Gram-positive organism was present in the last two cultures. In four successive cultures from November 29 to December 10 a Gram-positive diplococcus, growing in minute dew-drop colonies, was obtained, and in three successive cultures a yeast grew in very great numbers. It does not seem possible that any of these organisms was the cause of the cold. The yeast "infestation" seems to us of special interest. This organism practically never occurs in the mouth, and, as far as we know, never produces disease there, yet E. carried it in tremendous numbers for over a week. Some sort of a biological adaptation must have taken place, for experimental observations show that organisms otherwise disappear much more rapidly. The possibility that the alteration of the mucous membrane during the cold allows a temporary adaptation of various bacteria must be considered.

E.'s flora was studied from March 4 to April 25, 1920. It is interesting to compare the findings at that time with those present during his cold six months later. In the former series hemolytic influenza bacilli were present in two of ten cultures, and non-hemolytic influenza bacilli in four. During the recent series no non-hemolytic influenza bacilli were found. The transient flora last spring was less varied than during this winter. For details, see Case IX in the previous report (ref. 27).

CASE V.—M

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 9...	A few cols.	∞	40 cols. (hemolytic).	0	A few cols.	.....	Fifth day of cold. Moderate general symptoms. No complications. Slight mucoid nasal discharge. Throat slightly red.
Dec. 11..	A few cols.	∞	50 cols. (hemolytic).	0	0	....	Cold worse, but no complications.
Dec. 14..	Many cols. (three kinds).	∞	6 cols. (hemolytic).	0	0	.....	Well.
Jan. 8...	∞	∞	0	0	0	.....	Well.

Comment.—Clinically, the case is one of an uncomplicated cold. The normal flora was present throughout. Hemolytic influenza bacilli were obtained in three of four cultures. A few colonies of pneumococcus were obtained in the first culture but not thereafter.

The flora of this individual was studied about six months ago. At that time no hemolytic influenza bacilli were obtained, but non-hemolytic influenza bacilli were present in four of six cultures.

PROTOCOLS—CONTINUED

CASE VI.—L

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 1...	∞	∞	50 cols. (hemolytic).	0	0	∞ dew-drop cols. of a gram-pos. coccus. Many huge flat colonies of a gram-pos. streptococcus.	Second day of cold. Stuffiness in nose; throat sore. Slight malaise.
Dec. 9...	∞	∞	10 cols. (hemolytic).	0	0	A few huge flat colonies of a gram-pos. streptococcus. Culture from tracheal mucus; mixed flora—same as mouth.	Feels better. Tracheitis with sputum for several days.
Jan. 19..	∞	Many cols.	∞ (hemolytic).	0	0	.....	Well.

Comment.—Clinically, the case is one of a cold complicated by a tracheitis. The normal flora and also hemolytic influenza bacilli were constantly present. On the first culture innumerable colonies of a Gram-positive coccus were obtained. On the first two cultures an unusual non-hemolytic streptococcus growing in huge (3–5 mm.) flat colonies was obtained. Culture from the tracheal sputum showed no specific organism.

CASE VII.—T

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 12..	A few.	200 cols.	20 cols. (hemolytic). ∞ cols. (non-hemolytic).	0	0	Many colonies of a coarse Gram-pos. coccus. ∞ cols. of a minute Gram-pos. organism (pleomorphic bacillus?). 20 cols. Staph. albus. A few cols. of a Gram-pos. bacillus in chains.	Onset Nov. 10, began with irritation in nose and slight sore throat. Today feels very badly, fever and aching. Clear discharge from nose. Throat a little red. Tonsils removed.
Nov. 15..	∞	∞	3 cols. (hemolytic). Many cols. (non-hemolytic).	0	0	Many colonies of a coarse Gram-pos. coccus. A few cols. Staph. albus.	Cold still in active stage. Nose running, throat raw. Cough with muco-purulent expectoration.
Nov. 17..	A few.	∞	Many cols. (hemolytic).	0	0	∞ colonies of a Gram-pos. diplococcus. ∞ cols. of a minute Gram-pos. organism.	Better. Throat still raw. Moderate muco-purulent expectora-
Nov. 19..	Many cols.	∞	A few cols. (hemolytic). Many cols. (non-hemolytic).	0	0	.....	Improving, throat still raw. Less cough and sputum.
Nov. 30..	Many cols.	∞	50 cols. (hemolytic). Many cols. (non-hemolytic).	0	0	Many cols. of a Gram-pos. diplococcus. 10 cols. Staph. albus.	Well.
Dec. 13..	A few.	∞	6 cols. (hemolytic).	0	0	Many cols. of a Gram-pos. diphtheroid.	Well.
Jan. 19..	∞	∞	100 cols. (hemolytic).	0	0	A few cols. of a Gram-pos. diphtheroid.	Slight sore throat.

Comment.—Clinically, the case is one of a cold complicated by tracheitis. The normal flora as well as hemolytic influenza bacilli were constantly present. Non-hemolytic influenza bacilli were obtained in four of seven cultures. Various organisms were present as transients.

CASE VIII.—BL

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 21..	0	∞	0	0	0	Several hundred cols. of a coarse gram-pos. coccus; 15 cols. of a yellow staph.	Third day of cold. Nose stuffy, throat raw. Malaise and chilliness. For one day tracheitis with thick green purulent sputum.
Dec. 29..	∞	∞	0	0	0	A few cols. of a coarse gram-pos. coccus; 15 cols. of a yellow staph.	Practically well. Throat still a little raw, slight cough with mucoid sputum.
Jan. 8...	Several kinds.	∞	0	0	0	Many cols. of a yellow staph.	Well. Still very slight throat catarrh.
Sputum culture Dec. 21.	0	0	0	0	∞	Many cols. of a coarse gram-pos. coccus.	

Comment.—Clinically, the case is one of a cold complicated by a severe tracheitis. The normal flora was constantly present. This is the only case in the series in which hemolytic influenza bacilli were absent. Two organisms which are not normally present in the throat were obtained—a yellow staphylococcus, and a coarse Gram-positive coccus. From the sputum a pneumococcus was obtained in almost pure culture. This organism was clearly associated with the tracheitis.

PROTOCOLS—CONTINUED

CASE IX.—O

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 5...	A few cols.	∞	100 cols. (hemolytic).	0	0	.....	Onset Nov. 3. Fullness in nose and sneezing. To-day (3d day) head feels congested and nose is stopped up. Slight mucoid discharge. Throat not sore. Does not feel ill. Exam.—nasal m. m. red and swollen, large tonsils, pharynx injected.
Nov. 6...	A few cols.	∞	Many cols. (hemolytic).	0	0	.....	Condition unchanged.
Nov. 8...	A few cols.	∞	Many cols. (hemolytic).	0	0	.....	Condition unchanged.
Nov. 12..	∞	∞	20 cols. (hemolytic).	0	0	.....	Nose clear. Says left tonsil is sore, but it does not look remarkable.
Nov. 15..	0	∞	∞ cols. (hemolytic).	cols. β type.	0	.....	Says throat has felt very raw for three days. Looks a little red.
Nov. 16..	A few cols.	∞	∞ cols. (hemolytic).	∞ cols. β type.	0	.....	Throat quite raw and sore, looks a little red, no exudate. No fever, but feels chilly.
Nov. 19..	∞	∞	3 cols. (hemolytic).	0	6	20 large grey-green slightly hemolytic cols. of a long-chained streptococcus. Many minute tough grey colonies of a small Gram-pos. organism.	Throat still a little sore, but the patient is practically well. Throat looks normal.
Dec. 14..	Many cols.	∞	∞ cols. (hemolytic).	0	0	Many colonies of a Gram-pos. diphtheroid.	Cold well. Right side of throat has been sore from time to time.

Comment.—Clinically, the case is one of a cold complicated by tonsillitis. The normal flora and also hemolytic influenza bacilli were present throughout. On the tenth day, the patient developed a sore throat and coincidently innumerable colonies of a hemolytic streptococcus were obtained, which disappeared as the tonsillitis subsided.

CASE X.—R

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 8...	Many cols.	∞	0	0	Many cols. Type IV.	∞ cols. Staph. albus. Many cols. of a minute Gram-pos. organism. Sputum culture: Pneumococcus, Type IV (pure culture).	Cold began one week ago. Rhinorrhœa, slight sore throat, moderate general symptoms. On 4th day developed cough and sputum. Feels better now, but severe cough with thick green purulent sputum persists. On examination throat is slightly red.
Dec. 11..	Many cols.	∞	0	0	A few cols. Type IV.	∞ cols. Staph albus. Sputum culture: Pneumococcus, Type IV (pure culture).	Condition unchanged.
Dec. 14..	∞	∞	0	0	0	∞ cols. Staph albus. Sputum culture: Many cols. Staph albus. A few cols. M. catarrhalis. Many cols. of non-hemolytic strept.	Feels better. Cough much diminished. Only small amount mucoid sputum now.
Dec. 17..	Many cols.	∞	Several hundred cols. (hemolytic).	0	0	∞ cols. Staph albus. A few cols. of a minute Gram-pos. organism. Many dew-drop brownish cols. of a Gram-pos. coccus.	Feels well. No sputum. Persistent nasopharyngeal drip.
Dec. 20..	Many cols.	∞	Many cols. (hemolytic).	0	0	∞ cols. Staph albus. Many dew-drop brownish cols. of a Gram-pos. coccus.	Condition unchanged.
Jan. 4...	A few cols.	Many cols.	Many cols. (hemolytic).	0	0	∞ cols. Staph albus.	Condition unchanged.

Comment.—Clinically, the case is one of a cold, complicated by bronchitis. The normal flora was constantly present. From the bronchial pus a pneumococcus, Type IV, was isolated in pure culture. This was clearly associated with the complicating bronchitis. On the fourth culture hemolytic influenza bacilli were obtained and thereafter they were constantly present.

Innumerable colonies of a white staphylococcus were present throughout. As this is a distinctly abnormal finding, another complication was suspected. It was found that the patient had a chronic ethmoid infection for which surgical treatment had been advised by Dr. Crowe.

Results

With the knowledge previously gained of the normal throat flora as a background, the present cases were studied to determine any variations from the normal which might occur during the course of a cold, and to see if such variations might be interpreted in such a way as to shed light on the etiology of the disease.

In the uncomplicated cases the outstanding fact was this, that the flora differed in no fundamental way from that which obtains in healthy individuals. As in normal controls, non-hemolytic streptococci and Gram-negative cocci are constantly present. As in the normal, there is a shifting transient flora consisting of various organisms—diphtheroids, coarse Gram-positive cocci, minute Gram-positive organisms, white staphylococci and others. Two points in regard to this transient flora are, however, worthy of note. It is distinctly richer and more varied than that found in our series of controls, and in some cases organisms persisted for considerable periods of time—as in the case of the yeast infestation in E. A conceivable explanation of these findings is that the disturbance of the mucous membranes during the cold allows a general increase in the activity of bacterial growth on these surfaces. This fits in with the old theory that the cold, whether produced primarily by cold or by infection, leads to environmental changes which, as it were, light up the bacterial flora already present in the mouth. However, we feel definitely that none of these organisms can be the primary cause of the cold because their presence is too variable and inconstant, and the variety of them is great.

The almost constant presence of hemolytic influenza bacilli was of interest. These organisms were absent in only one case of the series. At first one was tempted to relate them to the cold in an etiological way, but it soon appeared that they could be nothing more than saprophytes, inasmuch as they were present in equal numbers in unaffected controls. Their frequency, however, illustrates one of the grave pitfalls of respiratory bacteriology.

The cases showing a clinical complication were of great interest, for here, in practically every instance, it was possible definitely to find that an organism which is not normally present was producing the complication. In O. a tonsillitis was associated with a hemolytic streptococcus, in B. and in R. bronchitis was associated with a pneumococcus, and in R. a sinus infection with Staph. albus.

Discussion

On clinical grounds the view was advanced that the common cold is an infectious disease analogous to influenza, featured by the frequent development of complications in the upper air passages such as sinus infections, tracheitis, and otitis. A review of the literature showed no convincing evidence that any known organism is the primary cause of the cold.

The cultural studies in the present report fail to show in uncomplicated cases any variation in the flora which would enable one to select any organism or group of organisms as the cause of colds. On the other hand, where clinical complications occurred pathogenic organisms were definitely associated with them.

We feel therefore that the primary cause of colds is probably an organism as yet unknown and certainly not one of the usual pathogens such as Streptococcus, Pneumococcus, B. influenzæ, or Staphylococcus. But the primary cold, whatever its final cause, alters the mucous membranes in such a way as to allow secondary bacterial invasion and consequent frequent development of local complications. The cultures clearly indicate that such complications are due to a variety of bacteria such as Pneumococcus, Streptococcus, and Staphylococcus.

In general it seems that the method of serial comparative study is necessary in working out the bacteriology of respiratory infections. Such a method allows one to pick out and interpret the significance of unusual organisms and also checks premature conclusions as to the etiological bearing of such organisms.

BIBLIOGRAPHY

1. Osler and McCrae: The Principles and Practice of Medicine. 8th ed., 1912, p. 382.

2. Fantus, B.: The Therapy of Catarrhal Fevers. Jour. A. M. A., 1920, **75**, p. 1694.

Hayes, Thomas: A Serious Address on Colds, etc. 4th ed., Dublin, 1786, p. 19.

4. Carpenter, J. G.: The Etiology, Pathology and Treatment of Acute Catarrh of the Upper Air Passages. Jour. A. M. A., 1889, **12**, p. 514.

5. Zwisohn: Bicarbonate of Sodium in the Treatment of a Common Cold. Medical Record, 1897, XLIX, 247.

6. Ewing, F. C.: Catching Cold, Its Cause and Prevention. Jour. A. M. A., 1897, **28**, p. 104.

7. Hitz, H. B.: Atmospheric Changes as a Direct Factor in the Etiology of Catarrhal Diseases of the Respiratory Tract. Laryngscope, 1896, I, 281.

8. Jack, G. N.: Meteorotellurgic Physiobiochemics of Coryza, Asthma, and Influenza. N. Y. State Jour. of Med., 1919, XIX, 269.

9. Schade, H.: Beiträge zur Umgrentzung und Klarung einer Lehre von der Erkaltung. Ztschr. f. d. ges. exp. Med., 1919, VII, 275.

10. Schade, H.: Untersuchungen in der Erkaltungsfrage. Muenchener Med. Wchnschr., 1919, LXVI, 1021.

11. *Ibid.* 1920, LVII, 449.

12. Miller, J. A. and Noble, W. C.: The Effects of Exposure to Cold upon Experimental Infection of the Respiratory Tract. Jour. Exp. Med., 1916, IV, 223.

13. Mudd, S. and Grant, S. B.: Reaction of Body Surface to Chilling. Jour. Med Research, 1919-20, XL, 55.

14. Grant, S. B., Mudd, S., and Goldman, A.: A Further Experimental Study on Excitation of Infections in the Throat. Jour. Exp. Med., 1920, XXXII, 113.

15. James, N.: Colds and the Prevention of Colds. Medical News (N. Y.), 1905, LXXXVI, 1123.

16. Haig, A.: The Etiology, Prevention and Treatment of a Common Cold. International Clinics, 1903, 135 LL, p. 167.

17. Walsh, J. J.: The Etiology of Colds. Med. News (N. Y.), 1903, LXXXII, 481.

18. Cecil, R. L.: The Laryngoscope, 1915, XXV, 97.

19. Voorhees, I. W.: Colds, their Cause and Cure. Amer. Med., 1917, ns., XII, 125.

20. Mackay, L.: Bacteriology of Chronic Nasal Catarrh. Brit. Med. Jour., 1919, XI, 159.

21. Tunnicliff, R.: Jour. Infec. Dis., 1913, XIII, 289.

22. Floyd, C.: Common Colds in Relation to Certain Micro-organisms, etc. Boston Med. and Surg. Jour., 1920, CLXXXII, 389.
23. Pfeiffer, H. and Ghon, L.: Ztschr. f. Klin. Med., 1902, XLIV, 262.
24. Kruse, W.: Die Erreger von Husten und Schnupfen. Muench. Med. Wchnschr., 1914, LXI, 1547.
25. Foster, G. B.: Etiology of Common Colds. Jour. A. M. A., 1916, LXVI, 1180.
26. Foster, G. B.: Etiology of Common Colds. Jour. Infec. Dis., 1917, XXI, 451.
27. Bloomfield, A. L.: J. H. H. Bull., 1921, XXXII, 33.

HEMOLYTIC INFLUENZA BACILLI

By T. M. Rivers and Erida L. Leuschner

(From the Department of Pathology and Bacteriology, The Johns Hopkins University)

Members of the pneumonia commission working at Fort Sam Houston in 1918 noticed, in throat cultures from patients with measles, colonies of Gram-negative bacilli surrounded by a zone of hemolyzed red blood cells. The incidence of hemolytic streptococci in throats was being studied at the time and the similarity in the types of hemolysis caused by these two organisms was striking enough to be of interest. Later some of these men, studying measles, influenza and pneumonia at Camp Pike, encountered this same Gram-negative hemolytic bacillus in throat cultures. They recognized that the types of hemolysis caused by this bacillus and by the hemolytic streptococcus were similar, but that the growths were different, the former making clear dew-drop colonies, the latter opaque, whitish colonies. They also noticed that the bacillus would not grow in meat-infusion broth.

Very little can be found in the literature about Gram-negative, non-motile, aërobic, hemolytic, hemoglobinophilic bacilli. Pritchett and Stillman [1] recovered an organism of this kind from throat cultures and called it "Bacillus X." Evidently at that time they did not consider it in the B. influenzæ group, as they say, "The colonies most readily confused on inspection with those of B. influenzæ were those of Micrococcus catarrhalis, Meningococcus, diphtheroids, and especially an unidentified organism called 'Bacillus X.'" In a previous paper on the biological classification of influenza bacilli, one of the authors,[2] after showing that B. influenzæ could be differentiated from B. pertussis culturally and that the former group could be sub-divided culturally, reported having studied some of the hemolytic hemoglobinophilic bacilli and having found that they, also, were not all alike culturally, and at that time suggested that they might be classified as hemolytic influenza bacilli. In a recent paper by Stillman and Bourn [3] all of the above statements have been confirmed and much valuable information has been added. Nevertheless they speak of non-hemolytic hemoglobinophilic bacilli as B. influenzæ and hemolytic hemoglobinophilic bacilli as "Bacillus X."

Pfeiffer [4] originally described a small, Gram-negative, non-motile, aërobic, hemoglobinophilic bacillus which he called the influenza bacillus. Then he found the pseudo-influenza bacillus. The distinction was made on morphology alone. Later he decided there was no real difference between the false and the true forms and that all influenza bacilli were alike. This view cannot be held at present for there is enough evidence to show that these bacilli are not all alike even in their cultural characteristics. In fact, all Pfeiffer did was to discover a new group of organisms and call them influenza bacilli. The question of hemolysis did not enter into the earliest descriptions of these bacilli. That point would have been hard to determine as they were first cultured by streaking bloody or purulent sputum on hardened agar plates and later by streaking a pure culture with blood on the surface of agar. From later descriptions it can be gathered that they were non-hemolytic, at least all bacteriologists considered them so. There is, however, a group of bacilli which coincides in every respect with the meager description given to influenza bacilli by Pfeiffer with the exception that they are hemolytic. The object of this paper is to show, if possible, that they belong in the large group of influenza bacilli just as hemolytic and non-hemolytic streptococci fall into the great group of streptococci and to suggest that bacteriologists in the future speak of hemolytic and non-hemolytic influenza bacilli.

In October, 1919, the throats of forty normal medical students were swabbed and from twelve (30 per cent) a Gram-negative, hemolytic, hemoglobinophilic bacillus was recovered. Later these cultures were lost, but during the epidemic of influenza in the early part of 1920, from 25 per cent of 52 throat cultures, 13 strains were isolated from influenza patients. None was isolated from the lungs or from blood cultures at autopsies, and so far as is known, they are not pathogenic for man. These strains have been studied but all have not been carried through, as some have died. However, five strains have been successfully carried through all the work and have been under constant observation for nine months.

All the throat cultures were made on 2 per cent fresh rabbit blood meat-infusion agar, pH 7.5. Both the hemolytic and the non-hemolytic groups grow well on this medium and they can be differentiated immediately, whereas it is impossible to do so on the oleate or cooked blood media. In a previous paper Rivers [5] showed that fresh human blood inhibited markedly the growth of B. influenzæ. Those were non-hemolytic. The same, however, is true of the hemolytic group and their growth is inhibited to such an extent by fresh human blood that it is impossible to use it in the work of isolation of these organisms with any degree of satisfaction. Fresh rabbit or cat blood, 2 per cent, can be used and as far as growth is concerned is just as satisfactory as the oleate or cooked blood media; moreover in other respects it is more satisfactory, since the hemolytic and non-hemolytic bacilli can be differentiated on the

original plates, giving the relative number of the two organisms present.

Naturally, the first question to be answered is, Are these bacilli hemoglobinophilic? Much work has been done to show that this is untrue of Pfeiffer's influenza bacillus and it is to be supposed that the same objections will be raised in regard to these hemolytic bacilli. Anyway, it can be said that one group is as hemoglobinophilic as the other. Pritchett and Stillman,[1] Stillman and Bourn [3] and one of us (Rivers)[2] have found them so. Meat-infusion agar or broth, pH 7.1-7.5, enriched with blood free ascitic fluid, is lacking in something necessary for growth, yet growth takes place in almost any kind of medium if a very little blood is added. The amount of blood necessary is very small and at times is hard to detect after it has been added. The hemolytic as well as the non-hemolytic bacilli show the phenomenon of symbiosis on unfavorable media and like the non-hemolytic ones will also grow for some generations on hemoglobin-free media in symbiosis with the proper organisms.

When 2 per cent rabbit blood meat-infusion agar plates were inoculated with these 13 strains, within 24 hours a zone of hemolyzed cells could be seen immediately around the streaks or the individual colonies. The cells were actually destroyed and the hemoglobin had diffused through the medium causing a deeper red color at the junction of the hemolyzed with the unhemolyzed portions of the plates. It was the beta type of hemolysis as applied to hemolytic streptococci. Some strains constantly formed wider zones of hemolysis than others but the type was always the same, the difference being merely one of degree. Hemolysis occurred in liquid as well as on solid media. After the cultures in liquid media were about a week old, long after the hemolysis had been completed, some would develop a greenish brown, others a dirty blackish brown color, and in some there was a fine whitish sediment sticking to the sides of the tubes, seen best at the top of the cultures.

Apparently a free hemolysin was demonstrated by growing the bacilli in "chocolate blood" meat-infusion broth. When the blood was coagulated and precipitated by heat a fairly clear medium was produced which could be used for the study of free hemolysin formation. Eight- to twelve-hour cultures were centrifuged till the supernatant fluid was clear. Half of the supernatant fluid was heated at 56° C. for 30 minutes and then to both portions 1 per cent washed rabbit cells were added and incubated at 37° C. for several hours. Slight hemolysis occurred in the unheated portion whereas none appeared in the heated one. Filtering the cultures through Mandler filters removed much of the hemolysin, but its presence was thought to have been demonstrated several times.

The addition of dextrose to the medium prevents hemolysis of red blood cells by hemolytic streptococci. Four strains of these hemolytic bacilli were grown on solid and in liquid media with and without dextrose to determine whether hemolysis could be influenced. The hemolysis produced by three of them was retarded and diminished in amount by the presence of dextrose; that produced by the fourth strain did not seem to be affected. All four strains fermented dextrose, but the fourth did so slowly, and hemolysis was not affected, while the others fermented the sugar fast enough to diminish the hemolysis but not fast enough to inhibit it completely as is the case with hemolytic streptococci. Dextrose should be omitted from all media which are used to show the presence or absence of hemolysis by members of the hemoglobinophilic group.

Pfeiffer's bacillus has been spoken of by many as a strict aërobe. In the seventh edition of the text-book by Park and Williams [4] it is called a facultative anaërobe. It seemed interesting to determine what relationship there is between the hemolytic and the non-hemolytic groups in regard to this matter. Meat-infusion agar with 2 per cent rabbit blood was inoculated with five of the hemolytic strains and one meningitic strain obtained from Dr. Wollstein. The cultures were put in a large vacuum jar, the air was exhausted (29-30 inches) three times, the jar being filled, between the evacuations, with hydrogen from a Kipp generator. After 24 hours' incubation the vacuum was tested and found to be satisfactory. The cultures were examined and a good growth was obtained in all. It seems from this that the hemolytic as well as the non-hemolytic bacilli are facultative anaërobes.

The colony formation of both groups, hemolytic and non-hemolytic, is almost identical, and if it were not for the hemolysis, it would be impossible in many instances to distinguish one from the other. Some of the hemolytic ones form large colonies which are more or less uniform in size; others grow in small uniform colonies, while still others show an irregularity in colony formation. Young colonies are clear and oval with smooth edges. Older colonies have brownish granular centers, and become checkerman, cone, or truncated cone-shaped, with irregular edges due to the formation of daughter colonies. Some are moist and soft, others somewhat tough, but all of them leave a shallow pit in solid media when a colony is moved. Streak cultures have lobate edges.

It is rather difficult to discuss the morphology of either the hemolytic or the non-hemolytic group, as this is rarely the same twice in succession. In general it may be said that the hemolytic bacilli are slightly longer, heavier, show squarer ends, and stain more deeply and regularly than the non-hemolytic ones. Some, however, are small cocco-bacilli and as far as morphology is concerned cannot be distinguished from those of the non-hemolytic group. No motility has been observed in 8- to 10-hour blood-broth cultures. All take the ordinary stains fairly well but irregularly, are distinctly Gram-negative, and do not form spores.

Blood-broth tubes inoculated with the 13 strains of hemolytic bacilli were incubated for varying periods of time, three to ten days. Then they were extracted with a small amount of ether. This ether extract was decanted and layered with Ehrlich's reagent. Three strains always gave a positive test for indol, the other ten were constantly negative.

Tubes of potassium nitrate blood-broth inoculated with the 13 strains were incubated for five days and then tested with the sulphanilic acid and naphthylamin reagent for nitrites. The uninoculated control tube gave a negative result, but all

of the inoculated tubes gave a positive test, a deep pink or reddish color showing the presence of nitrites.

Five of the hemolytic strains were grown in milk to which a little blood had been added. All produced alkali. Pritchett and Stillman [1] showed this previously. Many of the non-hemolytic ones, if not all, also produce alkali in blood-milk mixtures.

In another paper Rivers,[2] early in the work, stated that none of the hemoglobinophilic bacilli fermented certain sugars. This is incorrect, although he was unable at that time to demonstrate it. This point will not be taken up here but will be dealt with in a later paper. This much can be said; both the hemolytic and the non-hemolytic groups ferment certain sugars. This has been well shown by Stillman and Bourn.[3] There is nothing in the sugar fermentations to prevent the hemolytic group from being called influenza bacilli.

DISCUSSION

Thus far no reason has been found why the hemolytic group of these hemoglobinophilic bacilli should not be called influenza bacilli just as much as the non-hemolytic ones. There is no proof that some of the original organisms described by Pfeiffer as B. influenzæ did not belong to the hemolytic group. Very likely, using the methods described early by him, he worked with both groups and thought they were alike.

An interesting observation was made during the course of the work. One of the 13 strains, after cultivation for seven months on artificial media, lost completely its ability to hemolyze blood both on solid and in liquid media, while retaining all of its other cultural characteristics unchanged. Enough was known of its sugar fermentations and other cultural characteristics to exclude the possibility of a mistake. Any doubt is further excluded by the fact that the hemolytic stock strains were kept separate and transferred at a different time from the non-hemolytic ones. If the classification suggested, or implied, by Stillman and Bourn [3] be followed, the designations to be given to this strain would also imply fundamental differences in its character which its history denies. The organism, once a hemolytic hemoglobinophile, would be called "Bacillus X" of Pritchett and Stillman,[1] now, a non-hemolytic hemoglobinophile, should be called B. influenzæ. This is not believed to be the case. It has always been an influenza bacillus. Once it had many cultural characteristics, one of which was the ability to hemolyze red blood cells. Now it is the same bacillus except it has lost one of its many cultural characteristics, its power to produce hemolysis.

CONCLUSION

On account of the meager description given to the original B. influenzæ, especially concerning hemolysis, it seems best to regard both hemolytic and non-hemolytic aërobic, non-motile, Gram-negative, hemoglobinophilic bacilli as influenza bacilli and disregard any such confusing terms as "Bacillus X" which in itself represents not one organism but a group. After all, it makes very little difference what any of these bacilli, non-hemolytic or hemolytic, are called, so long as everyone recognizes that they belong to the same big group.

BIBLIOGRAPHY

1. Pritchett, I. W., and Stillman, E. G.: Jour. Exper. Med., 1919, XXIX, 259.
2. Rivers, T. M.: Bull. Johns Hopkins Hosp., Feb., 1920, XXXI, 50.
3. Stillman, E. G., and Bourn, J. M.: Jour. Exper. Med., 1920, XXXII, 665.
4. Pfeiffer, R.: Ztschr. f. Hyg., 1893, XIII, 357.
5. Rivers, T. M.: Bull. Johns Hopkins Hosp., May, 1919, XXX, 129.
6. Park, W. H., and Williams, A. W.: Pathogenic microörganisms, Phila. and New York, 7th edition, 1920, 457.

EXPERIMENTAL OBSERVATIONS ON BONE MARROW

By George B. Wislocki

(From the Department of Anatomy, Johns Hopkins Medical School)

The deposition of pigments in the bone marrow under physiological and experimental conditions has been studied by a number of investigators. Ponfick (1869) and Hoffmann and Langerhans (1869) observed the intracellular deposition of granules of cinnabar in the bone marrow after injecting it into the circulation of the living animal. They thought that the cells concerned in the phagocytosis of particulate matter in the bone marrow were lymphocytes. Cousin (1898) injected suspensions of carmine or litmus blue into animals and made the important observation that the pigment was phagocytosed by the endothelial cells lining the capillaries of the bone marrow. Ribbert (1904), using lithium carmine, confirmed Cousin's observations. Nasse (1876) and Quincke (1880) described the normal presence of pigment in the bone marrow of different animals, but it remained for Brass (1913) to demonstrate that this material is also stored within endothelial cells.

Finally, Evans (1915), using vital dyes such as trypan blue and pyrrhol blue, found that these also were deposited in the bone marrow. He observed, however, that although a large quantity of the dye was concentrated in the cytoplasm of endothelial cells, there were other phagocytic cells in the marrow—the so-called reticulum cells—whose connection with the endothelium is not clear. Recent experiments by Nagao (1920), who injected a suspension of carbon particles into the blood-stream of rabbits and guinea-pigs, confirm Evans' observation that, in the marrow, reticulum as well as endothelial cells participate in the phagocytosis of foreign particles.

The observation that the endothelial and reticular cells of the bone marrow phagocytose solid particles brought to them by the circulating blood led to the present experiment. It

was known that is was possible to inject into the blood stream of the living animal a pigment which would be phagocytosed by the marrow cells, and that after the animal was killed the bones of the entire body could be decalcified and the tissues cleared by the Spalteholz method. It was hoped that in this way the bone marrow of the entire body would be delineated by the presence of the opaque pigment in its cells.

The first series of experiments was performed on rabbits. The pigment used was a suspension of carbon particles, available in ordinary India ink. The ink, diluted one-half with distilled water, was injected into the ear vein of adult rabbits in doses of from 5 to 10 c. c. in two successive days. Newborn rabbits received 0.5 c. c. on two successive days. Two or three days after the last injection the animal was killed.

At autopsy the characteristic distribution of carbon particles in the body was observed. The carbon was confined practically to the liver, spleen, lungs, and bone marrow, to which tissues it imparted a gray or black appearance readily visible to the naked eye. On gross inspection the bone marrow appeared conspicuously black. The remaining tissues of the body were normal in color. Microscopic examination showed that carbon granules were abundantly present in the liver, spleen, lungs, and bone marrow. In the liver they were deposited in the Kupffer cells; in the spleen, in the reticulo-endothelial cells; in the lungs, in macrophages, possibly of endothelial origin; in the bone marrow granules were observed, for the most part intracellular, in the endothelium of the vessels and in the cells of the reticulum, although here and there granules were encountered lying free in the vascular channels.

It is interesting to note that the carbon particles, administered in the quantities named, produce no serious effects on the animal's health. The animals may be allowed to live for periods of months without showing any symptoms attributable to the foreign particles stored in the liver and other organs. Gross and microscopic examination of the tissues after a period of months reveals that the carbon deposited in endothelial cells has not diminished in amount nor produced any pronounced pathological changes. It appears that the inert foreign material is permanently stored in the endothelial cells without severe damage to the tissue, much as carbon is deposited in the lungs by inhalation.

The animals which were injected for clearing were sacrificed on the third or fourth day following injection. The bodies were skinned, eviscerated, and fixed in 10 per cent formalin. After fixation the bones were decalcified by placing the bodies in 5 per cent nitric acid until decalcification was complete. The specimens were then washed thoroughly, bleached with hydrogen peroxide, and dehydrated by alcohol. They were finally cleared by the method described by Spalteholz (1914), which consists in transferring them from absolute alcohol to benzol for 48 hours, then into several changes of oil of wintergreen and one part of benzyl benzoate, in which they are kept permanently. In the specimens prepared in this way the bones and tissues surrounding them become perfectly transparent, while the bone marrow stands out conspicuously as deep black patterns (Figs. 1 to 5).

The findings in the adult skeleton will be first described. Carbon was abundantly present in the cervical, thoracic, lumbar, sacral, and caudal vertebræ. It extended from the bodies of the vertebræ through the pedicles into the vertebral arches, whence it was continuous with deposits of carbon in the transverse and spinous processes. The carbon consequently completely outlined the shape of the vertebræ.

Carbon was deposited heavily and uniformly throughout the ribs. On close inspection the rib marrow appeared fenestrated. The sternebræ were also conspicuously black. The sixth segment of the sternum, the xiphoid process, exhibited an elongated black strip of marrow, to which a broad, flat plate of colorless cartilage was attached.

In the skull the deposition of carbon occurred in both membrane and cartilage bones. It was confined, however, to those bones which are in whole or in part cancellous. Thus, the occipital, posterior sphenoidal, anterior sphenoidal, interparietal, parietal, and frontal bones stood out prominently as a result of the black pigmentation between the outer and inner tables (Figs. 4, 6, 7, 8). In other bones, namely the squamosal, the petrotympanic, the maxillary, the zygomatic, and the mandible, in which the cancellous portions are restricted, pigmentation was scant. In the ethmoidal, inferior turbinate, premaxillary, nasal, lacrimal, palatine, and vomer bones no deposition of carbon granules was observed. The distribution of carbon granules in the bones of the skull is diagrammatically shown in Figures 6, 7, and 8.

The hyoid bone was deeply pigmented, as were also the lesser and greater cornua, two independent elements which articulate with it.

The deposition of carbon in the scapula was confined, as in the bones of the skull, to the cancellous regions. Thus, the head and neck of the bone, the axillary and superior borders, the scapular spine, the acromion and metacromion, were deeply pigmented. The vertebral border and the supraspinous and infraspinous fossæ were transparent. The clavicle, which in the rabbit, as in many other mammals, is an imperfectly developed bone, was visible as a narrow black line attached to the sternum by the sterno-clavicular ligament.

The humerus, radius, and ulna were completely outlined by a continuous, opaque black mass. The carpal and metacarpal bones and the phalanges of the digits contained only traces of carbon.

The coxal bone was clearly outlined as a heavy black structure. The femur, tibia, and fibula were deep black. The tarsal and metatarsal bones and phalanges contained traces of carbon.

In a three weeks' old rabbit the distribution of carbon differed somewhat from that in the adult (Fig. 5). The heaviest pigmentation occurred in the long bones of the extremities. The carpal, metacarpal, tarsal, and metatarsal bones, and the phalanges were deeply pigmented. In the long bones, in which the epiphyses and diaphyses were still ununited, carbon was visible in the cancellous portions of the epiphyses as well as in the shafts of the bones. The coxal bone contained three distinct masses of carbon corresponding to the ilium, ischium, and pubis. The distribution of carbon in the scapula

resembled that in the adult. The clavicles were visible as two thread-like lines. The hyoid bone contained a tiny black center. In the skull only traces of carbon were visible in the as yet only partially ossified occipital, parietal, frontal, superior maxillary, zygomatic, temporal, posterior sphenoidal, anterior sphenoidal, and mandibular bones.

In a rabbit one week after birth carbon particles were abundantly phagocytosed and stored in those bones in which ossification had commenced. Thus the separate ossification centers of the vertebræ stood out as well-defined black masses. The ribs each contained a prominent band of black marrow, and there was abundant carbon in the sternebræ. The bones of the extremities, in which ossification had commenced, were filled with carbon-colored marrow. In the skull the deposition was limited to portions of the basioccipital, the exoccipital, the supraoccipital, the basisphenoidal, the presphenoidal, the squamosal, and the mandibular bones.

The different bones were examined microscopically.. Clusters of blood-forming cells occurred fairly uniformly throughout the cancellous portions and shafts of all the bones and consequently in the rabbit a distinction between yellow and red marrow could not be made. Particles of carbon were abundantly present in the cytoplasm of reticulum cells and in the endothelial cells lining the marrow capillaries. The distribution of carbon particles was found to coincide closely with that of the hemopoietic tissue. In the adult animals carbon was found nowhere else than in the marrow of the bones, but in the developing animals a few granules of carbon were observed in some of the osteoblasts in the regions of the epiphyseal lines.

From these observations it will be seen that the bone marrow of the rabbit contains cells which are extremely phagocytic toward inert particulate material afloat in the blood stream. Some of these are endothelial cells which line the vascular channels of the marrow, others are reticulum cells which form the supporting tissue for the blood-forming elements of the marrow. This phagocytic power is present at birth. Microscopic examination reveals that the distribution of these phagocytic cells in the bones closely coincides with that of the blood-forming elements. The cleared tissues of rabbits injected during life with carbon particles give us an accurate picture of the gross distribution of the marrow. The amount of marrow in flat bones is in direct proportion to the amount of cancellous or spongy structure which the bone possesses.

As the experiments described above were confined to the rabbit, it was deemed important to observe the behavior of the bone marrow of other mammalia towards particulate matter, for the purposes of comparison. To this end several dogs, cats, and guinea-pigs were similarly injected with suspensions of carbon. In the dog and cat carbon was not grossly visible in the marrow, and on microscopic examination only an occasional carbon particle could be discovered in the endothelial cells. In the guinea-pig the marrow appeared in the gross definitely blackened by the presence of the carbon, but not to the same extent as in the rabbits receiving corresponding injections. Microscopically the marrow of the guinea-pig was found to contain numerous carbon-laden phagocytic cells, less numerous, however, than in the rabbit.

There exists, therefore, a difference in the ability of the bone-marrow cells in different mammalia to phagocytose and store particles of carbon. In the cat and dog the storage of inert particulate matter, so far as can be determined by quantitative observation, is confined to the liver, spleen, and lungs; in the guinea-pig the bone marrow plays an additional though subordinate rôle; while in the rabbit the material is distributed equally between the liver, spleen, lungs, and bone marrow.

The method herein described of observing the distribution of inert particles in cleared bones may prove valuable in the study of the pathology of bones. It would be of interest to use this method in the experimental study of healing fractures and in experimentally produced disturbances of bone during development and growth.

REFERENCES CITED

Brass, H.: 1913. Ueber physiologische Pigmentablagerung in den Kappillarenendothelien des Knochenmarks. Arch. f. mikr. Anat., LXXXII, 61.

Cousin, G.: 1898. Notes biologiques sur l'endothelium vasculaire. Compt. rend. Soc. biol., p. 454-456.

Evans, H. M.: 1915. The macrophages of mammals. Am. J. Physiol., XXXVII, 443.

Hoffmann, F. A., and P. Langerhans: 1869. Ueber den Verbleib des in die Cirkulation eingeführten Zinnobers. Arch. f. path. Anat. u. Physiol., XLVIII, 304.

Nagao, K.: 1920. The Fate of India Ink Injected into the Blood. Jour. Infect. Dis., XXVII, 527.

Nasse: 1876. Ueber das Vorkommen eisenhaltiger Körner im Knochenmark. Marburger Sitzungsberichte.

Ponfick, E.: 1869. Studien über die Schicksale körniger Farbstoffe im Organismus. Arch. f. path. Anat. u. Physiol., XLVIII, 1-54.

Quincke, H.: 1880. Zur Pathologie des Blutes. Arch. f. klin. Med., XXV, 567; *Ibid.*, XXVII, 193; *Ibid.*, 1883, XXXIII, 22.

Ribbert, H.: 1904. Die Abscheidung intravenös injizierten gelösten Karmins in den Geweben. Ztschr. f. allgem. Physiol., IV, 201-214.

Spalteholz, W.: 1914. Ueber das Durchsichtigmachen von menschlichen und tierischen Präparaten. Leipzig.

DESCRIPTION OF PLATE

FIG. 1.—Lateral view of thorax of a rabbit decalcified and cleared by Spalteholz's method, showing distribution of carbon in the ribs.

FIG. 2.—Anterior view of thorax of a rabbit decalcified and cleared by Spalteholz's method, showing distribution of carbon in the sternebræ.

FIG. 3.—Anterior extremity of a rabbit, showing the distribution of carbon in the bone marrow. The specimen has been decalcified and cleared by the Spalteholz method.

FIG. 4.—View of a decalcified, cleared skull of a rabbit, showing the deposition of carbon granules in the marrow.

FIG. 5.—Decalcified, cleared rabbit, 3 weeks of age. The animal had received two injections of a carbon suspension (India ink) intravenously and was sacrificed 48 hours after the second injection.

FIG. 6.—Diagram of dorsal view of skull of an adult rabbit. The black dots show the exact distribution of carbon granules in the bone marrow. (The outlines of the bones of the skull in Figs. 6, 7, and 8 are after Bensley, "Practical Anatomy of the Rabbit," 1910, and Gerhardt, "Das Kaninchen," 1909.)

FIG. 7.—Diagram of ventral view of a skull of an adult rabbit, the black dots indicating the deposits of carbon in the marrow.

FIG. 8.—Similar diagram of mandible.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 5.

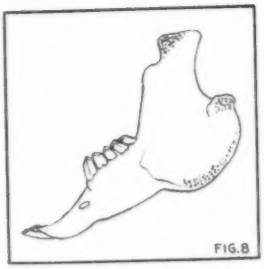

FIG. 8

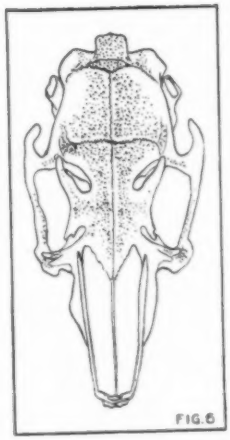

FIG. 6

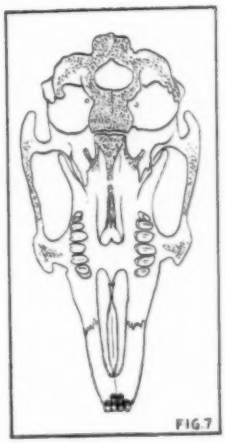

FIG. 7

FIG. 4.

THE RESIGNATION OF DR. WILLIAM SYDNEY THAYER

Dr. Frank J. Goodnow, President Johns Hopkins University, Baltimore, Md.

7, April, 1921.

Dear President Goodnow.—Something over three years ago, when the question arose as to my assuming the direction of the medical department, I expressed the opinion that this responsibility might more wisely be entrusted to one who could look forward to a longer tenure of office.

You know, I think, how deep and sympathetic has been my interest in the efforts which the university has been making to improve the standards of medical education and to broaden the opportunities for the study of the art of medicine and of its underlying scientific problems. You are further aware, I am sure, of my unreserved approval and sympathy with those improvements of organization and methods which, in latter years, we have been enabled to initiate through the generous assistance of the General Education Board—improvements which are but the natural evolution from the advances introduced by Osler over thirty years ago.

In the developments which are sure to follow within a few years, it is important that the medical department be under the direction of a man specially qualified by character, training and experience; it is, however, especially desirable that he be one who may reasonably look forward to twenty or twenty-five years of activity in the execution of his ideals and his conceptions.

For this reason I have felt, and more than once have said, that I regarded myself as undertaking these responsibilities for a limited period—until there might appear the men or man obviously qualified for the task.

It is my conviction that this moment has arrived.

I can imagine few opportunities more delightful or congenial or stimulating to the physician of high ideals than those offered by the Professorship of Medicine at The Johns Hopkins University. I am, however, convinced that for the furtherance of the cause of sound medical education and for the development and expansion of those general plans on which we have so happily entered—in the success of which I am deeply concerned—I am convinced that the moment has come to hand over the direction of the department of medicine to a younger man.

I beg leave, therefore, to offer you my resignation as Professor of Medicine at The Johns Hopkins University to take effect on July 1, 1921.

In so doing pray let me assure you that, as in the past, so in the future, my services, in whatever capacity or to whatever extent they may be desired, will always be at the disposition of the university.

Believe me, dear Dr. Goodnow,

Very sincerely yours,

(Signed) W. S. Thayer.

MINUTE ON THE RESIGNATION OF DR. THAYER

In accepting the resignation of Dr. William S. Thayer as Professor of Medicine in The Johns Hopkins Medical School and Physician-in-Chief to The Johns Hopkins Hospital, the members of the boards of trustees of the university and of the hospital, together with the members of the Medical Faculty of the university and of the Medical Board of the hospital, desire to record their deep appreciation of the long and distinguished services rendered to the university and hospital by Dr. Thayer. He began his work as Assistant Resident Physician on Dr. Osler's wards in 1889, and, except for the period of his important war-activities, has continued uninterruptedly in posts of ever higher responsibility until the present time.

In his care of patients in the hospital, in his teaching of students and assistants, in his original studies, especially those on malaria and on diseases of the heart and blood vessels, in his papers and addresses distinguished not only for their contents but also by their clarity, forcefulness and elegance of style, and in his personal relationships with the public, as well as with the profession both in this country and abroad, he has attained an international reputation, and has added notably to the prestige of the hospital which he has so faithfully served; while his rare qualities both of head and of heart make him stand out as the highest type of clinician of his time.

Though Dr. Thayer feels that the direction of the medical clinic should now be assumed by a somewhat younger man, his associates are glad to know that the hospital and university may still avail themselves of his diagnostic skill, his rich experience and his valued counsel.

BOOKS RECEIVED

American Climatological Association. Transactions. For the Years 1918-1919. Volumes XXXIV and XXXV, 1918 and 1919. 8°. 192 and 294 pages. Printed for the Association. Lancaster, Pa.

The Harvey Lectures. Delivered under the Auspices of The Harvey Society of New York. 1917-1918; 1918-1919. Series XIII and XIV. 1920. 8°. 295 pages. J. B. Lippincott Company, Philadelphia and London.

American Ophthalmological Society. Transactions. Fifty-fifth annual meeting. Volume XVII. 1919. 8°. 741 pages. American Ophthalmological Society. Philadelphia.

U. S. Treasury Department. Public Health Reports. Issued weekly by the United States Public Health Service. Containing information of the current prevalence of disease, the occurrence of epidemics, and related subjects. Volume 33, parts 1 and 2, 1918. Government Printing Office, Washington, 1919 and 1920.

U. S. Treasury Department. Public Health Reports. Issued weekly by the United States Public Health Service. Containing information of the current prevalence of disease, the occurrence of epidemics and related subjects. Volume 34, part 1, 1919. Government Printing Office, Washington, 1920.

U. S. Department of Commerce. Bureau of the Census. Financial Statistics of States. 1919. 4°. 119 pages. Government Printing Office, Washington, 1920.

U. S. Department of Commerce. Bureau of the Census. Birth Statistics for the Birth Registration Area of the United States. 1918. Fourth annual report. 1920. 4°. 312 pages. Government Printing Office, Washington.

Physiology and Biochemistry in Modern Medicine. By J. J. R. Macleod, M. B. Assisted by Roy G. Pearce, A. C. Redfield, and N. B. Taylor and by others. Third edition, with 243 illustrations, including nine plates in colors. 1920. 8°. 992 pages. C. V. Mosby Company, St. Louis.

Physiology and Pathology of the Cerebrospinal Fluid. By William Boyd. 1920. 8°. 176 pages. MacMillan Company, New York.

The Link Between the Practitioner and the Laboratory. A Guide to the Practitioner in his Relations with the Pathological Laboratory. By Cavendish Fletcher, M. B., B. S., Lond., M. R. C. S., L. R. C. P., and Hugh McLean, B. A., B. C. Cantab., D. P. H., Camb., M. R. C. S., L. R. C. P. 1920. 8°. 91 pages. Paul B. Hoeber, New York.

Practical Vaccine Treatment. For the General Practitioner. By R. W. Allen, M. A., M. D., B. S., Late Capt. N. Z. M. C. 1920. 8°. 308 pages. Paul B. Hoeber, New York.

Diabetes. A Handbook for Physicians and Their Patients. By Philip Horowitz, M. D. With twenty-seven text illustrations and two colored plates. 1920. 8°. 196 pages. Paul B. Hoeber, New York.

Public Health Laboratory Work (Chemistry). By Henry R. Kenwood, C. G., M. B., F. R. S. Edin. D. P. H., F. C. S. Seventh edition, with illustrations. 1920. 8°. 420 pages. Paul B. Hoeber, New York.

The School of Salernum. Regimen Sanitatis Salernitanum. The English Version by Sir John Harington. History of the School of Salernum by Francis R. Packard, M. D. And a Note on the Pre-history of the Regimen Sanitatis by Fielding H. Garrison, M. D. 1920. 8°. 215 pages. Paul B. Hoeber, New York.

Refraction and Motility of the Eye. With Chapters on Color Blindness and the Field of Vision. By Ellice M. Alger, M. D., F. A. C. S. With one hundred and twenty-five illustrations. Second revised edition. 1920. 12°. 394 pages. F. A. Davis Company, Philadelphia.

Short Talks on Personal and Community Health. By Louis Lehrfeld, A. M., M. D. With introduction by Wilmer Krusen, M. D., LL. D. 1920. 12°. 271 pages. F. A. Davis Company, Philadelphia.

Electro-Therapy: Its Rationale and Indications. By J. Curtis Webb, M. A., M. B., B. C. (Cantab.). With six diagrams. 1920. 12°. 90 pages. P. Blakiston's Son & Co., Philadelphia.

A Course of Lectures on Medicine to Nurses. By Herbert E. Cuff, M. D. F. R. C. S. Seventh edition, with 29 illustrations. 1920. 12°. 257 pages. P. Blakiston's Son & Co., Philadelphia.

Massage: Its Principles and Practice. By James B. Mennell, M. A. M. D. B. C. (Cantab.), etc. With an Introduction by Sir Robert Jones, K. B. E., C. B., F. R. C. S. Maj.-Gen. A. M. S. Second edition. With 167 illustrations and two appendices. 1920. 8°. 536 pages. P. Blakiston's Son & Co., Philadelphia.

A Text-Book of Pathology. By W. G. MacCallum. Second edition, thoroughly revised. 1920. 8°. 1155 pages. W. B. Saunders Company, Philadelphia and London.

The Story of the American Red Cross in Italy. By Charles M. Bakewell. 1920. 8°. 253 pages. Macmillan Company, New York.

Florence Nightingale. By Eleanor Francis Hall. (With four illustrations and a map.) Pioneers of Progress, Women. Edited by Ethel M. Barton. 1920. 12°. 84 pages. Society for Promoting Christian Knowledge, London. Macmillan Company, New York.

Consultations pour les Maladies des Voies Digestives. Par Dr. Gaston Lyon. 1920. 8°. 360 pages. Masson et Cie, Paris.

Orthopædics for Practitioners. An Introduction to the Practical Treatment of the Commoner Deformities. By Paul Bernard Roth, M. B., Ch. B. (Aberd.), F. R. C. S. (Eng.). 1920. Edward Arnold, London.

Diathermy in Medical and Surgical Practice. By Claude Saberton, M. D. With thirty-three illustrations. 1920. 12°. 138 pages. Paul B. Hoeber, New York.

Practical Bacteriology, Blood Work and Animal Parasitology. Including Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes. By E. R. Stitt, A. B., Ph. G., M. D., Sc. D., LL. D. Sixth edition, revised and enlarged with 1 plate and 177 other illustrations containing 637 figures. 1920. 8°. 633 pages. P. Blakiston's Son & Co., Philadelphia.

Backwaters of Lethe (Some Anæsthetic Notions). By G. A. H. Barton, M. D. With illustrations. 1920. 12°. 151 pages. Paul B. Hoeber, New York.

Some Conclusions on Cancer. By Charles Creighton, M. D. With 124 illustrations in the text. 1920. 8°. 365 pages. Williams and Norgate, London.

Occupational Affections of the Skin. Their Prevention and Treatment with an Account of the Trade Processes and Agents Which Give Rise to Them. By R. Prosser White, M. D., Ed., M. R. C. S., Lond. Second edition, with twenty-four plates comprising twenty-eight figures. 1920. 8°. 360 pages. Paul B. Hoeber, New York.

The Johns Hopkins Hospital Bulletins are issued monthly. They are printed by the LORD BALTIMORE PRESS, Baltimore. Subscriptions, $4.00 a year (foreign postage, 50 cents), may be addressed to the publishers, THE JOHNS HOPKINS PRESS, BALTIMORE; single copies will be sent by mail for fifty cents each. Single copies may also be procured from the BALTIMORE NEWS CO., Baltimore.